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(57) Abstract

Organic compounds and mixtures thereof and chelates of organic compounds with metal salts which show pharmacological activity in the treatment of cancers and non-malignant tumors, and against bacteria, viruses and fungi. More particularly, mixtures and chelates of metal salts with organic compositions wherein the metal portion is a multivalent metal halide. The compositions of this invention are less toxic than an equivalent amount of at least one separate component thereof. This invention also relates to the use of zinc chloride as a potentiator of organic compounds useful for the treatment of human tumors, and further relates to the use of specific organic compounds for topical treatment of human tumors. Methods of treatment of cancers and non-malignant tumors with the compositions described above are also disclosed, as are methods of treatment of bacterial, viral and fungal infections, and methods of selective debriding and skin ulcers.

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PHARMACOLOGICALLY ACTIVE COMPOUNDS AND MIXTURES THEREOF, ORGANIC COMPOSITIONS AND METAL SALTS

Field of the Invention

This invention relates to pharmacologically active compounds and mixtures thereof and chelates of selected organic compounds as defined herein with and without metal salts. The mixtures and chelates are useful in the treatment of cancers and non-malignant tumors and as antibacterial, antiviral and antifungal agents. The invention also relates to certain organic lignans and aliphatic acids useful as topical pharmaceuticals against tumors.

Background Art

A number of metal salts have been reported to have antitumor activity. The organo-platinum coordination compounds, e.g. cisplatin, are probably the best known. Additionally, colloidal lead phosphate, alkali arsenites, various organo-arsenicals and copper, nickel and cobalt butylphthalate complexes have been utilized in the treatment of human cancers. Zinc chloride and other escharotics have been utilized as treatments for human cancers, but, except as used in controlled applications

in chemosurgical techniques, such uses generally have not received favorable consideration from the medical profession. Zinc chloride, including zinc chloride mixed with an extract from podophyllum, has been used as a fixative in chemosurgical removals of cancerous skin growth with layers of the growths being removed within a short period of time after each application of the fixative. Zinc chloride has also been used in combination with plant materials such as bittersweet root and blood root to cure skin lesions and/or tumors. However, the prior art contains no suggestion that the presence of an organic compound renders a composition containing zinc chloride more effective, more selective, or less toxic in destroying diseased or damaged tissue and promoting healing thereafter.

A number of articles by Mohs on the treatment of skin cancer (e.g., F. E. Mohs, M.D., and M. F. Guier, PhD., "Pre-Excisional Fixation of Tissues in the Treatment of Cancer in Rats," Cancer Research, 1:49-51, 1941) disclose the use of a mixture of zinc chloride in an amount of about 40% to 50% by weight of the preparation combined with stibnite and a plant extract of _Sanguinaria canadensis_ to chemically kill and fix cancerous tissue _in situ_ prior to microscopic excision of the tumor cells. Mohs' article mentions that other plant extracts such as those of _Phytolacca decandra_, _Podophyllum peltatum_ or _Inula helenium_ may also be used as the agglutinant in his preparation to keep the fixative solution from separating as it stands in the jar. The article does not teach or suggest that the plant extract has activity beyond its stated function to keep the preparation suspended.

U.S. 2,344,830, a patent granted to Mohs for the above composition, specifically discloses that his preparation is for conditioning diseased tissue for removal by surgery, and is not used as, nor intended as, a cure for diseased tissue.

U.S. 4,315,916 to Jonas S. Likens, discloses the use of a compound derived from dried bittersweet root bark (Solanum dolcamarum) combined with zinc chloride to form a salve applied topically to remove unwanted skin growths. The bittersweet root extract is identified only by an approximate empirical formula, ($C_{15}H_{25}O$) and through its physico-chemical properties.

Booth, et al., in "Metabolic Effects of Zinc in Intact Cells - Comparative Studies of Zinc Chloride and the Zinc Chelate of Kethoxal bis (thiosemicarbazone)," 20 Biochem. Pharm, 3109 (1971), compares the cytotoxic effects on mice bearing transplanted intraperitoneal sarcoma 180 ascites cells of intraperitoneal injections of 20 mg./kg. zinc chloride and 50 mg./kg. of a zinc chelate of Kethoxal bis (thiosemicarbazone), a known anticancer agent, and found the zinc chelate to be significantly more cytotoxic than zinc chloride alone. The zinc chelate used was not a mixture of the organic with zinc chloride.

Ladanyi Patent No. 4,406,881 discloses the use of a mixture of a mineral acid salt of benzophenanthridine alkaloid and zinc chloride as an antimicrobial agent, and states that, *in vivo*, a combination of these two ingredients was more effective than either ingredient alone against dental plaque-forming microorganisms.

Although such escharotics as zinc chloride have long been known to destroy tissue, it has not been known that these escharotics are active in combination with organic compositions as described below to selectively destroy tumor tissue in concentrations lower and less toxic than the threshold concentrations at which such escharotics act alone to destroy tissue.

Nordihydroguaiaretic acid (NDGA) has been investigated for potential antibacterial activity and has been

found to possess such activity in vitro. Chaparral tea, which contains NDGA, has also been reported in the last decade to be useful in the treatment of human cancer; however, subsequent studies conducted as a result of the alleged anticancer activity have concluded that the tea is not beneficial in the treatment of cancer. Extracts from Larrea have also been utilized in the treatment of Bovine eye cancer.

Burk, D., et al., "Hydrogen Peroxide, Catalase Glutathione Peroxidase, Quinones, Nordihydroguaiaretic Acid, and Phosphopyridine Nucleotides in Relation to X-Ray Action on Cancer Cells," Rad. Res. Supp., 3:212-246 (1963) discloses the use of NDGA as a cancer antimetabolic agent in vitro and in vivo, using for the in vivo test an intraperitoneal injection of 400 mg. NDGA (hydroquinone form) per kg, a dosage highly toxic to the host. NDGA was thought to inhibit glycolysis, accounting for its selectivity for tumor cells as opposed to normal cells which do not conduct much glycolysis.

Gisvold, et al., "Lignans from divaricata," J. Pharm Sci., 63:1905-07 (1974) discloses that NDGA in combination with ascorbic acid is effective in vivo against Ehrlich ascites tumor in mice, and suggests that similar activity might be shown by dihydroguaiaretic acid, norisoguaiacin, 3'-dimethoxyisoguaiacin, or partially demethylated dihydroguaiaretic acid.

Smart, C.R., et al., "Clinical Experience with Nordihydroguaiaretic Acid," Rocky Mountain Med. J. (November, 1970) discloses the successful self-treatment by an 85-year old male of malignant melanoma with two cups per day of chaparrel tea (containing NDGA) for several months. This article also reports negative NDGA screenings by the Cancer Chemotherapy National Service Center for sarcoma 180, mammary adenocarcinoma 755, and leukemia L1210 in mice. Tests of 59 humans with incurable malignancies, using chaparrel tea or pure NDGA at 250 mg.

to 3000 mg. per day by mouth, showed 4 significant regressions. A significant number of cases showed tumor stimulation.

None of the foregoing articles disclose preparations of NDGA for topical application effective for tumor reduction.

Many organic compounds have been found to have anti-tumor effects; however it has not previously been known that zinc and other metal ions are useful as potentiators and enhancers for these compounds in producing antitumor effects.

Zinc acetate (1.2%) in combination with erythromycin (4%) has been successful for the topical treatment of acne. See "Topical Erythromycin, Zinc Equal of Oral Tetracycline in Acne," Skin & Allergy News, p. 5, Vol. 14, No. 9, 1983. However, zinc chloride as a potentiator of organic agents for the treatment of such infections has not previously been known.

A. Breathnach, et al., in "Ultrastructural and Biochemical Observations on the Effect of 4-hydroxy-anisole plus Tyrosinase on Normal Human Melanocytes and Kerotocytes in Tissue Culture," Br. J. Cancer, Vol. 47, p. 813-822, 1983, describes the use of 4-hydroxyanisole plus tyrosinase for destruction of melanocytes in vitro.

M. Nazzaro-Porro, et al., "Effect of Azelaic Acid on Human Malignant Melanoma," The Lancet, 1109-11 (May 24, 1980), discloses the effectiveness of C_8 to C_{14} di-carboxylic acids for the topical treatment of cutaneous hyperpigmentary disorders such as chloasma, toxic mela-noderma, lentigo maligna, and malignant melanocyte. Malignant melanomas were treated with a 15% azelaic acid cream twice daily for 16 weeks. Patients were also treated orally. No toxic effects were observed, and tumor reduction occurred in all cases. The article disclosed that azelaic acid is a tyrosinase inhibitor.

M. Nazzaro-Porro, et al., "Effect of Dicarboxylic Acids on Lentigo Maligna," J. Invest. Dermatol., 1979, 72/6 (296-305) discloses the use of a 15% azelaic acid ointment to successfully affect abnormally active or structurally disordered melanocytes in lentigo maligna.

M. Nazzaro-Porro, et al., "Local Treatment of Lentigo Maligna with Azelaic Acid," Arch. Dermatol. Res. (Germany, West), 1981, 271/2 (197-203) describes the use of azelaic acid in an ointment to treat lentigo maligna. Results were not convincing in clinical aspects.

M. Nazzaro-Porro, et al., in "Beneficial Effect of 15% azelaic acid cream on acne vulgaris," Br. J. Dermatol., Vol. 109, pp. 45-48, 1983 describes the successful use of azelaic acid cream to treat acne.

H.E. Willshaw, et al., "Azelaic Acid in the Treatment of Ocular and Adnexal Malignant Melanoma," Br. J. Opthalmol., 1983, 67/1 (54-57) describes the unsuccessful use of azelaic acid both locally and systemically in treating malignant melanoma lesions of the eye and its adnexa.

A.S. Breathnach, et al., "Effect of Dicarboxylic Acids on Normal Human Melanocytes in Culture," Br. J. Dermatol, Vol. 99/Suppl. 16 pp. 19-20, 1978 reports that dicarboxylic acids have a stimulatory effect on melanocytes of an original culture generation which may result in destruction of melanosomes, but do not prevent growth of, or cause damage to second generation.

German Patent DE 2817133 dated November 2, 1978, to M. Nazzaro-Porro, (corresponding to Canadian Patent No. 1,137,873 submitted herewith) discloses the use of azelaic acid and other dicarboxylic acids having 7 to 13 carbon atoms for the treatment of hyperpigmental dermatoses.

None of the foregoing art suggests or discloses that dicarboxylic acids may be potentiated or enhanced in their antitumor activity by multivalent metal salts, that

monocarboxylic acids might be effective, or that the organic acids might be effective against non-hyperpigmentary disorders such as human breast tumors. Taken as a whole, the cited art teaches away from, as much as suggests, the use of dicarboxylic acids even for the treatment of hyperpigmentary skin disorders.

Additionally, many organic compositions have been investigated for their potential biological and pharmacological activities. A variety of activities have been reported including antiviral activities for some flavonoids, antimicrobial activities for some phenols and antitumor activities for some lignans and some phenols. However, much of this work has been conducted in vitro at a cellular level with different conclusions being drawn by different investigators. In fact, a recent article in J. Nat. Prod., 42:85-91 (1975), which collated the results of 217 flavonoids tested in the screening program of the National Cancer Institute, concluded not only that no correlation can be drawn between KB cytotoxicity screens and animal screens for antitumor activity of flavonoids, but also that flavonoids do not warrant further investigation as antitumor agents.

Finally, both metal compounds and many organic compositions, including phenolic compositions, have been tested for various antimicrobial activities. Many of these compounds do exert such activity.

Some of the compounds and compositions of this invention are discussed in co-pending applications Serial Nos. 365,784, 436,444 and 436,425, all commonly assigned to the assignee hereof. The contents of said applications are fully incoporated herein by reference.

Summary of the Invention

This invention relates to organic compounds and mixtures thereof, with and without metal salts, and to organo-metallic chelates, which show pharmacological activity in the

treatment of cancers and non-malignant tumors. Preferably, the metal salts are zinc salts, and preferably the chelates are zinc chelates. The mixtures and chelates of this invention are generally more effective and less toxic than effective doses of the separate components thereof.

The compositions of this invention are useful in the treatment of bacterial, viral and fungal infections, and for the selective debriding of skin ulcers. In addition, they are useful for the healing of lesions, acne, warts and inflammatory disorders.

This invention also relates to the use of metal ions, e.g. zinc, as enhancers and potentiators of organic compositions useful for treating tumors.

This invention further relates to methods of treatment of cancers and non-malignant tumors, to methods of treatment of bacterial, viral and fungal infections, and to selective debriding of skin ulcers with the compositions described above.

Detailed Description of the Preferred Embodiments

I. Organic Compounds and Escharotics.

Escharotics such as zinc chloride and other metal halides owe their activity to their ability in sufficient concentrations to indiscriminantly destroy living tissue with which they are placed in contact. These escharotics do not selectively destroy diseased tissue, such as tumor tissue, to the exclusion of healthy tissue. When sufficient concentration of the escharotic to be effective in eradicating a tumor is placed in contact with the tumor, it will also destroy any healthy tissue with which it comes in contact. It has now been discovered that when such escharotics are combined with the organic compositions described herein, the combinations are effective for the eradication of tumors even though the concentration of the escharotic is less than that which would be effective by itself. Also, it has been found that when organic compositions which are active alone to reduce

tumor sizes are used, less of the organic compositions are needed for activity when used in combination with subactive concentrations of escharotic.

The fact that the organic compositions and the escharotics enhance or extend the activity of each other means that lesser concentrations of toxic escharotics and organic compositions need be used to obtain pharmacological activity. Furthermore, with proper selection of the organic components, greater selectivity for tumor tissue may be achieved than with escharotic alone.

The multivalent metal salts of this embodiment are zinc, trivalent chromium, yttrium, divalent cobalt, platinum trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals. Generally, the highest oxidation state of a metal is preferred over lower oxidation states thereof. The most preferred metal salts are zinc salts, more preferably halides, with zinc chloride being the most preferred zinc halide. Escharotic metal salts including zinc chloride comprise the most highly preferred class of metal salts. A broader preferred class of metal salts comprises zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium salts. Within this class, the halides are preferred, and the chlorides most preferred. Zinc salts including nitrates, sulfates, acetates and halides also comprise a preferred class. Another, broader contemplated class comprises salts of all the metals above listed except the rare earth metals, preferably the halides thereof, and more preferably the chlorides thereof.

A wide range of organic compounds are useful for the reduction of tumors in combination with metal salts as defined herein. A mixture is provided comprising one or more of the above-described escharotics and one or more organic compounds as defined herein.

A most preferred embodiment of this invention utilizes meso-nordihydroguaiaretic acid (NDGA) as the organic component of the mixture and zinc chloride as the metal salt, the mixture being contained in a suitable pharmaceutical carrier. For topical use, carriers such as creams, ointments and solutions are provided. Polyethylene glycols of varying molecular weights are examples of suitable carriers. A preservative such as butylated hydroxytoluene (BHT) may be added as well as chelating agents such as ethylenediaminetetraacetic acid (EDTA).

For topical application the mixture may comprise about 1-30 weight percent zinc chloride, and about 1-18 weight percent nordihydroguaiaretic acid, with optional minor portions of ethylenediaminetetraacetic acid, and butylated hydroxytoluene, in a suitable carrier such as a mixture of stearyl alcohol and polyethylene glycol, with major portions of said polyethylene glycol having a molecular weight of around 400, and minor portions thereof having a molecular weight of about 3000 to 4000, said polyethylene glycols being present in proportions such that they provide a consistency suitable for topical application.

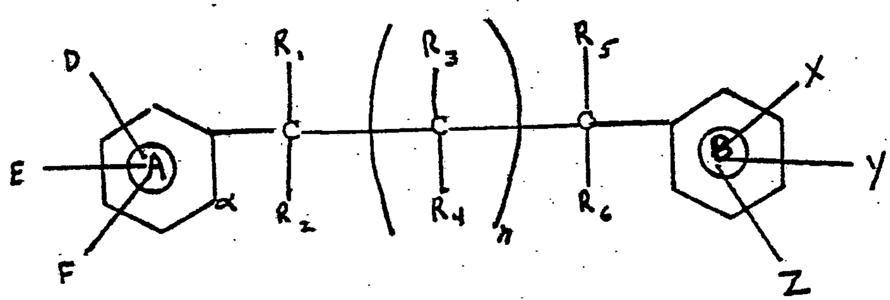
A typical mixture comprises about 4.60% NDGA, about 29.8% zinc chloride, about 14.7% ethylenediaminetetraacetic acid, about 1.1% BHT, about 0.50% stearyl alcohol, about 18.3% purified water, about 26.4% polyethylene glycol having an average molecular weight of about 400 and about 4.5% polyethylene glycol having an average molecular weight of about 3350. A further preferred mixture contains NDGA and zinc chloride in the proportions defined above, but does not contain edetic acid or BHT. These compositions may be prepared by any means known to the art to obtain uniform and stable mixtures.

Other typical compositions comprise: (1) about 4.6% NDGA, about 1.1% BHT, about 0.49% ethylenediamine-tetraacetic acid and about 1.0% zinc chloride, with the balance comprised of water and polyethylene glycols; (2) about 4.6% NDGA, about 1.1% BHT, about 2.47% ethylene-diaminetetraacetic acid and about 5.0% zinc chloride, with water and polyethylene glycols; and (3) about 4.6% NDGA, about 1.1% BHT, about 4.93% ethylenediaminetetra-acetic acid and about 10.0% zinc chloride with water and polyethylene glycols. Compositions (1), (2), and (3) without ethylenediaminetetraacetic acid or BHT are also preferred mixtures. The foregoing compositions may be made up with differing viscosities, depending on whether they are intended for topical use, injection, or other means of administration, by adjusting the relative amounts of polyethylene glycol 400 and polyethylene glycol 3350.

Another typical composition comprises the above proportions of NDGA, $ZnCl_2$, BHT and ethylenediaminetetra-acetic acid in a slow-release ointment formula which releases zinc chloride at a slower rate than NDGA.

A preferred preparation for sustained-release topical use contains NDGA at a concentration of between about 5 and about 15% and zinc chloride at a concentration of between about 5 and about 30%, and releases zinc ions at an average rate of between about 0.5 and 1 weight percent per hour, while releasing NDGA at a rate propor-tional to its concentration.

In general, many of the compositions according to the invention are of the general formula:



wherein, D,E,F,X,Y,Z, may be H;OH; O-Alkyl or O-Acyl optionally substituted with hydroxy, alkoxy, substituted amino, carbalkoxy, or carboxy;

R_1-R_6 may be H; lower alkyl or lower alkoxyl optionally substituted with hydroxy, alkoxy, substituted amino, carboxyl, or carbalkoxyl; hydroxy; carbonyl; alkoxy; atyl; atalkyl;

n may be 0 to 5;

any of the aromatic rings in the molecule may contain up to 3 substituents from the following list: hydroxy; alkenoxy; alkyl, alkoxy or alkanoyl optionally substituted by hydroxy, alkoxy, substituted amino, carboxy, or carbalkoxy; CF_3; halo; carboxy; carbalkoxy; cyano; hydroxymethyl; sulfonic acid; sulfonamido; aminosulfonyl (i.e. - $NHSO_2R$); nitro; alkoxycarbonyloxy; aminocarbonyloxy; aroyloxy; aralkanoyloxy; heteroaroyloxy; glycosidyloxy; and

any two phenolic groups may be joined together by the following groups: CH_2, CH_2–, CH_2–, HOP(=O), Alkyl-O-P(=O), R_2NP(=O)

either of the rings A or B may be replaced by cyclohexyl, napthyl, tetrahydronapthyl, pyridyl, piperidinyl, quinolinyl, indanyl, indenyl;

any of the groups R_1 to R_6 may be joined together to form together with the other carbons to which they are attached, a 5, 6, or 7 membered ring optionally in - terrupted by an oxygen atom, or containing an oxygen atom and a carbonyl substituent, or containing a carbonyl substituent;

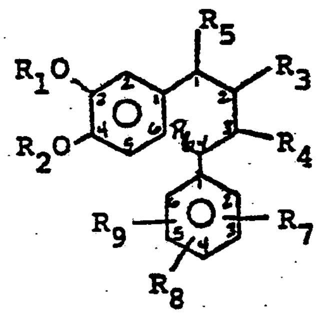
any of the groups R_3 to R_6 may be joined to ring A to form with it a 5, 6, or 7 membered ring;

any of the carbons in the chain between rings A and B, may be attached by a bond to the α position on ring A to form a 5, 6, or 7 membered ring.

More specifically, organic compounds useful in embodiments of the invention comprise:

A. Bridged Phenolic Compounds.

1. Catecholic butanes of the formula:



I.

where R_1 and R_2 are independently H; 1-12 alkyl; 1-12 alkenyl; 1-12 alkoxy; 1-12 alkenoxy; $(CO)_n$ $(CH_2)_m$ $(CO_2)_p$ R_a, where n=0-1, m=1-4, p=0-1, and R_a is independently H, 1-12 alkyl, and 1-12 alkenyl; and glycoside moieties and R-substituted glycoside moieties wherein any of the hydroxyl hydrogens thereof may be replaced by R, with R being independently 1-2 alkyl, and 1-2 alkoxy; and taken together are methylene;

R_7, R_8 and R_9 may be attached to any separate location C_1-C_6 of their benzene ring, and are independently H; O; OR_1 (with R_1 defined as above); and when R_7 and R_8 or R_8 and R_9 are adjacent, taken together they may be methylene;

R_3 and R_4 are independently H, CH_3, C_2H_5, CHO and COOH; and

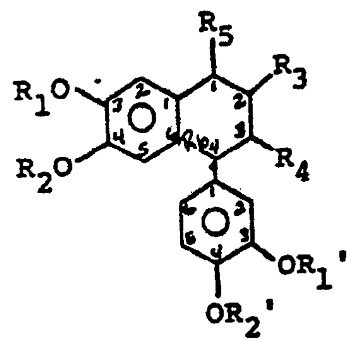
R_5 and R_6 are independently H, OH, OCH_3 and O.

A class of useful compounds of this invention corresponding to Formula I comprises those listed below exemplifying Formula II as well as the following: 3,4,2',5'-quatrahydroxy-1,4-diphenylbutane; 2',3',4',3,4-pentahydroxy, 1,4-diphenylbutane; 3',4',5', 3,4-pentahydroxy, 1,4-diphenylbutane; and 1-(3,4-dihydroxyphenyl), 4-phenylbutane.

Also included within the scope of this invention are those having trifluoromethyl substituents in place of

methyl substituents, e.g. 1-(3,4-dihydroxyphenyl)-4-(3,5-ditrifluoromethylphenyl) butane.

2. Preferred compounds of Formula I correspond to the formula:



II.

where R_1-R_6 are as defined in Formula I, and OR_1' and OR_2' are defined as R_1 and R_2, but may vary independently of R_1 and R_2.

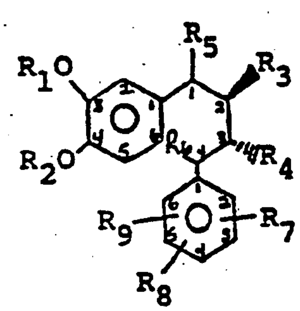
A preferred class of useful compounds of this invention are nordihydroguaiaretic acid and its analogs of Formula II comprising: nordihydroguaiaretic acid (1,4-bis-(3,4-dihydroxyphenyl) butane); dihydroguaiaretic acid (1,4-bis-(3-methoxy,4-hydroxyphenyl), 2,3-dimethyl-butane); nordihydroguaiaretic acid tetraacetate (1,4-bis(3,4-diacetoxyphenyl),2,3-dimethylbutane); nordihydro-guaiaretic acid propionate (1,4-bis-(3,4-dipropyloxy-phenyl), 2,3-dimethylbutane); nordihydroguaiaretic acid glycoside (1-(3-hydroxy, 4-glucaryl-O-phenyl), 4-di-hydroxyphenyl, 2,3-dimethyl-butane or 1-(4-hydroxy, 3-glucaryl-O-phenyl), 4-dihydroxyphenyl, 2,3-dimethyl-butane); nordihydroguaiaretic acid glycoside tetraacetate (1-(3-hydroxy, 4-tetraacetoxyglucaryl-O-phenyl), 4-di-hydroxyphenyl, 2,3-dimethylbutane or 1-(4-hydroxy, 3-tetraacetoxyglucaryl-O-phenyl), 4-dihydroxyphenyl, 2,3-dimethylphenylbutane); nordihydroguaiaretic acid diphenoxyacetic acid diethyl ether (1,4-bis (3-dihydroxy) 4-diethylcarbonylmethoxyphenyl), 2,3-dimethylbutane or 1,4-bis (4-dihydroxy, 3-diethylcarbonylmethoxyphenyl), 2,3-dimethylbutane); nordihydroguairetic acid triphenoxy-acetic acid diethylether (1-(3-hydroxy, 4-ethylcarbonyl-

methoxyphenyl), 4-(diethylcarbonylmethoxyphenyl), 2,3-dimethylbutane or 1-(4-hydroxy, 3-ethylcarbonylmethoxyphenyl), 4-(diethylcarbonylmethoxyphenyl), 2,3-dimethylbutane); nordihydroguaiaretic acid tetraethylhemisuccinate (1,4-bis(tetraethylhemisuccinylphenyl), 2,3-dimethylbutane; nordihydroguaiaretic acid tetramethylether diol (1,4-bis (3,4-dimethoxyphenyl) 2,3-dimethyl, 1,4-dihydroxybutane); nordihydroguaiaretic acid dimethylene ether dione (1,4-bis (3,4-dimethylenedioxyphenyl) 2,3-dimethyl, 1,4-dioxobutane); nordihydroguaiaretic acid tetramethylether dione (1,4-bis (3,4-dimethoxyphenyl), 2,3-dimethyl, 1,4-oxobutane).

A broader preferred class includes desmethyl nordihydroguaiaretic acid (1,4-bis (3,4-dihydroxyphenyl) butane); desmethyl nordihydroguaiaretic acid tetramethylether (1,4-bis (3,4-dimethoxyphenyl) butane, and other desmethyl analogs of the compounds of the preferred class listed above.

Also included within the scope of this invention are compounds corresponding to Formula II terminating in one or more nitro groups, e.g. nordihydroguaiaretic acid tetramethyl carbamate.

3. Included within the scope of this invention are stereoisomers of the above compounds in which R_3 and R_4 are not H, and are in d,l-conformation, it being understood that Formula I includes such compounds as well as those having a meso conformation. Specifically, such steroisomerism occurs at positions 2 and 3 of the butane chain:

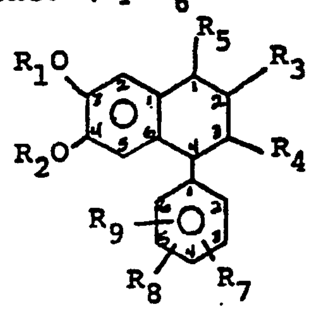


III.

(R_1-R_9 are as defined in Formula I above except that R_3 and R_4 are not H).

A preferred class of such compounds comprises d,l-forms of the compounds of Formula II exemplified by d,l-nordihydroguaiaretic acid and the d,l-isomers of the nordihydroguaiaretic acid analogs listed above as a preferred class. A broader class of such compounds comprises the d,l-isomers of the compounds listed above as exemplifying Formula I.

4. Also included within the scope of this invention are tetralins analogous to the compounds of Formula I wherein the phenyl group attached to C_1 of the butane chain is also attached (by C_6 thereof) to C_4 of the butane chain:

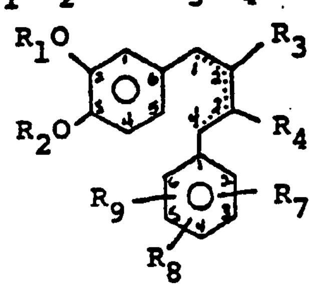


IV.

where R_1-R_5 and R_7-R_9 are as defined in Formula I above.

A preferred class of such compounds comprises tetralin analogs of the compounds of Formula II, exemplified by norisoguaiacin and tetralin analogs of nordihydroguaiaretic acid and the analogs thereof listed above as preferred classes. A broader class of such compounds comprises tetralin analogs of the compounds of the broader classes listed above.

5. Also included within the scope of this invention are catecholic butenes analogous to the compounds of Formula I wherein double bonds may occur at C_1-C_2, C_2-C_3, C_3-C_4, or at both C_1-C_2 and C_3-C_4:



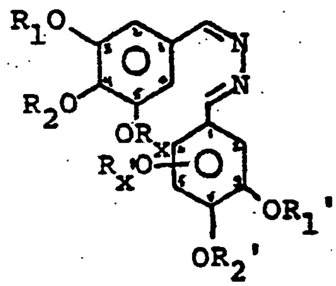
V.

where R_1-R_9 are as defined in Formula I above.

A preferred class of such compounds comprises butene analogs of the compounds of Formula II exemplified by 1,4-bis-(3,4-dimethylenedioxyphenyl) 1,3-butene and 2,3-bis (3,4-dimethoxybenzylidene) succinic acid, and butene analogs of the compounds listed above as preferred classes of Formula II. A broader class of such compounds comprises 1-(3,4-diacetoxyphenyl)-4-phenyl-buta-1,3-diene; and 1-(3,4-dihydroxyphenyl)-4-phenylbutadiene and analogs thereof in which the substituents at the 3 and 4 position of the phenyl may be, independently, OH, and OR wherein R is C_1-C_4 alkyl and/or alkoxy, or taken together, the substituents are methylenedioxy. A broader preferred class comprises the foregoing together with other butene analogs of the compounds listed above as broad classes of Formula I.

Also included within the scope of this invention are compounds having trifluoromethyl substituents rather than methyl substituents, e.g. 1-(3,5-ditrifluoromethylphenyl)-4-(3,4-dimethoxyphenyl)-1-butene; and compounds having nitro substituents, e.g. 1-(3,5-dinitrophenyl)-4-(3,4-dimethoxyphenyl)-1-butene.

6. Also included within the scope of this invention are 1,3-butene analogs of the compounds of Formula V in which C_2 and C_3 of the butane chain are replaced by N:

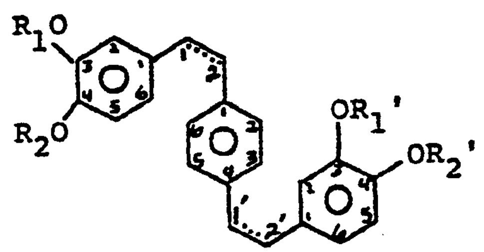


VI.

wherein R_1, R_2, R_1' and R_2' are defined as in Formula II above, and R_x and R_x' are defined as R_1 and R_1' respectively, and when $R_x'O$ and $R_2'O$ are adjacent they may be, taken together, methylene.

A preferred class of such compounds includes vanillin azine and syringaldezine, and catecholic analogs thereof wherein all but the 3' and 4' hydroxy substituents of one phenyl group may be replaced, independently with R, wherein R is H, 1,4 alkyl, or 1-4 alkoxy, and two adjacent such substituents taken together may be methylenedioxy.

7. Also included within the scope of this invention are 1,4-bis phenethylbenzenes and 1,4-bis styrylbenzenes of the formula:



VII.

wherein R_1, R_2, R_1' and R_2' are as defined in Formula II above, and there may be double bonds independently at 1-2 and 1'-2' of the chains.

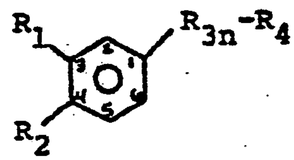
A preferred class of such compounds comprises 1,4-bis-(3,4-dihydroxyphenethyl) benzene; 1,4-bis-(3,4-dimethoxyphenethyl) benzene; 1,4-bis-(3,4-dihydroxystyryl) benzene; and 1,4-bis-(3,4-dimethoxystyryl) benzene.

8. Also included within the scope of this invention are catecholic propanes, pentanes, pentenes, hexanes and hexenes analogous to the compounds of Formula I, but having 3, 5- or 6-member carbon bridges between the phenyl groups rather than 4-member chains.

A preferred class of such compounds comprises 1,6-bis-(3,4-dihydroxyphenyl) hexane and other hexane analogs of the compounds listed above as preferred classes of Formula II. A more preferred class of such compounds comprises those having no substituents on the hexane bridge other than the two phenyl groups at positions 1 and 6.

B. Monophenolic Compounds.

1. Phenolic acids and acidic anhydrides selected from the group consisting of compounds of the formula:



VIII.

wherein R_1-R_2 are independently H, OH, 1-12 alkyl, 1-12 alkenyl, 1-12 alkoxy, 1-12 alkenyoxy, 1-12 alkylcarboxy, 1-12 alkenylcarboxy, or, taken together, are methylene dioxy;

$n = 0$-1

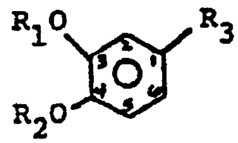
R_3 = 1-12 alkyl, 1-12 alkenyl, hydroxy-1-5-alkyl, hydroxy-1-5-alkenyl; oxy-1-5-alkyl; oxy-1-5 alkenyl, or oxo-1-5-alkyl, oxo-1-5 alkenyl; and

R_4 is an acid moiety, a 1-5 alkyl ester moiety, a 3-6 carbon dicarboxylic acid moiety, or a 3-6 carbon dicarboxylic acid anhydride moiety.

A preferred class of such compounds comprises 3,4-dihydroxybenzoic acid; ethyl 3,4-dihydroxybenzoate; cinnamic acid; mandelic acid; p-hydroxycinnamic acid; 3,4-dihydroxycinnamic acid; 3,4-dihydroxyphenylacetic acid; 4-hydroxy, 3-methoxycinnamic acid; 2-(3,4-dimethoxybenzylidene) succinic acid; and 2-(3,4-dimethoxybenzylidene) succinic anhydride.

Also included within the scope of this invention are compounds in which R_4 is an amine acid moiety. A preferred compound of this type is 3-(3,4-dimethoxyphenyl) propylamine, N-formic acid, N-acetic acid and its hydrochlorides and hydrobromides. Also included within the scope of this invention are compounds wherein R_1 and R_2, rather than being in the 3 and 4 positions, as shown in Formula VIII, are rotated to other adjacent positions. A preferred compound of this type is 2,3-dihydroxybenzoic acid.

2. Substituted catecholic compounds of the formula:



IX.

wherein R_1 and R_2 are independently, 1-12 alkyl, 1-12 alkenyl, or taken together are methylene; and

R_3 is 1-12 alkyl, 1-12 alkenyl, formyl, hydroxy-1-12-alkyl, hydroxy-1-12-alkenyl, oxo-1-12-alkyl, or oxo-1-12-alkenyl.

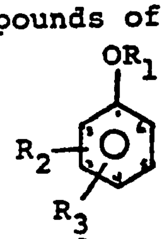
A preferred class of such compounds comprises 4-methyl catechol; 4-tertbutyl catechol; 3,4-dimethoxyphenyl ethanol; 3,4-dihydroxybenzaldehyde; vanillin; 3,4-dimethoxyacetophenone; and 3,4-methylenedioxypropiophenone.

Also included within the scope of this invention are compounds in which R_3 is an amine moiety. Preferred compounds of this type are 3,4-dihydroxybenzylamine and 3-(3,4-dimethoxyphenyl) propylamine, their hydrochlorides and hydrobromides.

Also included within the scope of this invention are compounds in which the phenyl group or substituents bear one, two or more simple substituents, including halo, nitro, sulfato amino, and the like. A preferred compound of this type is 5-nitrovanillin.

Also included within the scope of this invention are compounds wherein R_1O and R_2O, rather than being in the 3 and 4 positions, as shown in Formula IX, are rotated to other adjacent positions, and alkyl portions of R_3 are unbranched. Preferred compounds of this type are 2,3-dihydroxybenzaldehyde and 3-propyl catechol.

3. Phenolic compounds of the formula:



X.

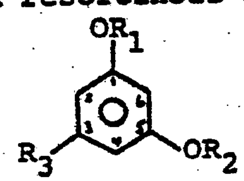
wherein R_1 is H or CH_3; and

R_2 and R_3 are independently H and 1-12 alkyl.

A preferred class of such compounds comprises phenol; 2-tertbutylphenol; 3-tertbutylphenol; 4-tertbutylphenol; thymol; and 2,3-dimethylphenol.

Also included within the scope of this invention are compounds in which the phenyl group or alkyl substituents bear one, two or more simple substituents including halo, nitro, amino sulfato, and the like. A preferred class of compounds of this type comprises picric acid; o-anisidine; 2-aminophenol; and pentafluorophenol.

4. Substituted resorcinols of the formula:



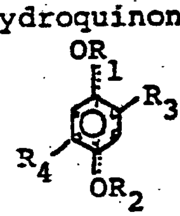
XI.

wherein R_1 and R_2 are independently H or CH_3; and

R_3 is 1-12 alkyl.

A preferred class of compounds of this type comprises orcinol; 4-ethyl resorcinol; and olivetol.

5. Quinones and hydroquinones of the formula:



XII.

wherein R_1 and R_2 are independently H and CH_3; and

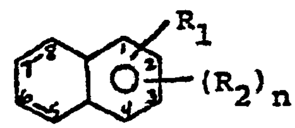
wherein R_3 and R_4 are independently H and OH.

A preferred compound of this type is hydroquinone. Another preferred class includes both hydroquinone and 2,5-dihydroxy-p-benzoquinone.

Also included within the scope of this invention are compounds wherein the phenyl group and/or alkyl substituents bear one, two or more simple substituents including halo, nitro, amino, sulfato and the like. A preferred compound of this type is chloranil.

C. Multinuclear Ring Compounds.

1. Naphthalenic compounds bearing oxygen-containing substituents of the formula:



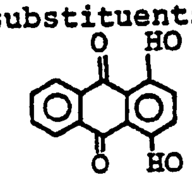
XIII.

wherein n is 0-1;

R_1 and R_2 are independently OH, CHO, and COOH.

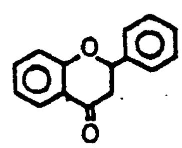
A preferred class of such compounds includes 2,3-dihydroxynaphthalene; 1-naphthaldehyde; and 2-naphthaldehyde.

2. Flavones, flavanones, coumarins, quinizarins, ellegic acids, and purpurogallins, bearing 0-2 substituents per ring, said substituents being independently 1-12 alkyl, hydroxyl, 1-12 alkoxy, formyl, carboxyl and oxosubstituents.



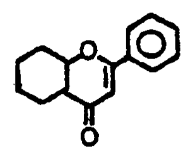
Quinizarin

XIV



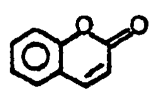
Flavone

XV



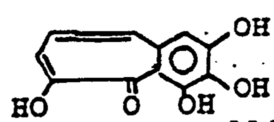
Flavanone

XVI



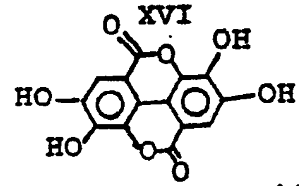
Coumarin

XVII



Purpurogallin

XVIII



Ellegic Acid

XIX

A preferred class of such compounds comprises flavone; flavanone; quercetin; 4-methyl esculetin; quinizarin; ellegic acid and purpurogallin trimethyl ether.

Also included within the scope of this invention are compounds wherein the rings or alkyl substituents bear one, two or more simple substituents including halo, nitro, amino, sulfato, and the like. A preferred compound of this type is calcein blue.

A further contemplated class of compounds represented by the above formulae comprises flavonoid compounds, both agylcones and glycosides, such as flavones, isoflavones, anthocyanins and flavans, and pharmaceutically acceptable salts thereof, with cations as defined above.

Examples of flavones are:
flavanol (3-hydroxyflavone); (3,5,7-trihydroxyflavone); (3,3'4'-trihydroxyflavone); (3,4',5-trihydroxyflavone); datiscetin (2',3,5,7-tetrahydroxyflavone); fisetin (3,3',4',7 tetrahydroxyflavone); (3,3',4'-trimethoxy-7-hydroxyflavone); (3,3',4',7-tetramethoxyflavone); morin 2',3,4',5,7-pentahydroxyflavone); (8-hydroxykaempferol) kaempferol (3,4',5,7-tetrahydroxyflavone); quercetin (3,3',4',5,7-pentahydroxy-flavone); quercetagetin (3,3',-4',5,6,7-hexahydroxyflavone); quercetin 3,3'-dimethylether; quercetin-7,3'-dimethylether; quercetin 3'-methylether; kaempferol (3,4',5,7-tetrahydroxyflavone); kaempferol-3,7-dimethylether; kaempferol 3-methylether; kaempferol 7-methylether; kaempferol 3,4'-dimethylether; quercetin 3,7,3',4'-tetramethylether; quercetin 3,7,3'-trimethylether; quercetin 7,3',4'-trimethylether; quercetin 3,7-dimethylether; luteolin 7,3'-dimethylether (3',4',5,7-tetrahydroxyflavone); luteolin 3'-methylether; apigenin 7-methylether (4',5,7-trihydroxyflavone); rutin (quercetin-3 rutinoside) 3,3'4',5,7-pentahydroxyflavone-3-rutinoside; chrysin (chrysoeriol 6,8-9-C-glucoside) 5,7-dihydroxyflavone; isoquercetin 3,3',4',5,7-pentahydroxyflavone-3-glucoside; kaempferol 3-O-rhamnosylglucoside (3,4',5,7-tetrahydroxyflavone); rhamnetin-3-o-rhamnosylglucoside (3,3',4',5-tetrahydroxy-7-methoxyflavone); myricetin (3,3',4',5,5'7-hexahydroxyflavone) (dihydromyricetin 3',5-dimethylether); hesperetin (herbacetin) 3,7-dimethylether; (3',5,7-trihydroxy-4'-methoxyflavanone); quercimeritrin $C_{21}H_{20}O_{12}$; 3,3',4',5,7-pentahydroxyflavone-7-D-glucoside [gossypitrin $C_{21}H_{20}O_{13}$] 3,7,3'-trimethylether.

Examples of flavans include catechin (3,3',4',5,7-flavanpentol); gallocatechin; polydine; adzelechin, eupatilin and 4'-demethyl-eupatilin.

Examples of anthocyanins include delphinidin (3,3',-4',5,5',7-hexahydroxyflavilium); leuteoliniden; cyaniden (3,3',4,5,7-pentahydroxy flavylium); peonidum (3,4',5,7-tetrahydroxy-3'-methoxyflavylium); myrillidin; and enidin.

D. Aliphatic Acids, Aldehydes and Alcohols.

Active aliphatic compounds of this invention are preferably unbranched chains containing oxygen and having four to twelve carbon atoms.

A preferred class of aliphatic acids of this invention includes dicarboxylic acids having four to twelve carbons. Most preferred is the class comprising adipic acid, azelaic acid, lauric acid and oxydiacetic acid.

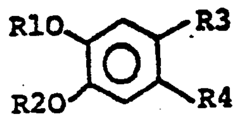
A preferred aliphatic alcohol of this invention is lauryl alcohol.

A preferred aliphatic aldehyde of this invention is octyl aldehyde.

Also included within the scope of this invention are aliphatic cyanides having four to twelve carbon atoms. A preferred compound of this type is n-octyl cyanide.

E. Other Compounds

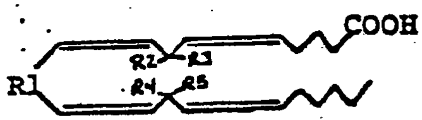
Other useful compounds in accordance with the invention include compounds of the formula:



XXI.

where R1 and R2 are, independently, H and CH_3, or taken together, are CH_2;
where R3 is a dienoic 4-12 fatty acid moiety; and
where R4 is a dienoic 4-12 fatty acid moiety or a 4-12 mono or dialkene moiety.

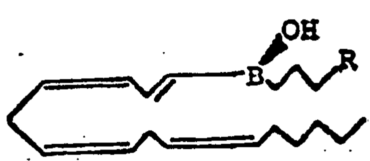
Preferably R1 and R2 are H; and R3 is deca-1(E),4(E)-dienyl or octa-2(Z)-enyl; and R4 is nona-9-carboxy,1(E)-4(E)-dienyl or deca-10-carboxy,2(Z),5(Z)dienyl;



XXII.

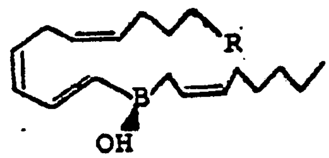
where R1 is CH_2, O, NH, CF_2 or CHF; and
where R2, R3, R4 and R5 are, independently, F and H.

Preferably R1 is CH_2 and R2 and R3 are F and R4 and R5 are H; or R1 is CH_2 and R2 and R3 are H and R4 and R5 are F; or R1 is CF_2 and R2, R3, R4 and R5 are H; or R1 is O and R2, R3, R4 and R5 are H; or R1 is NH and R2, R3, R4 and R5 are H. Where only one fluorine is present, it is preferred that this fluorine be such as to form an L-fluoro-compound;



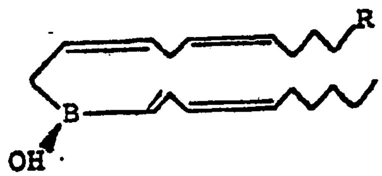
XXIII.

Where R is COOH, CH_3, or CHO.



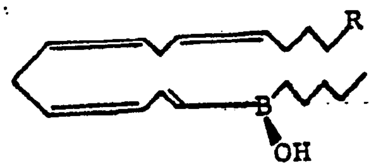
XXIV.

Where R is COOH, CH_3 or CHO ;



XXV.

Where R is COOH, CH_3 or CHO ;



XXVI.

Where R is COOH, CH_3 or CHO .

Compounds illustrative of Formula XXI are: 4-(deca-1(E),4(E)-dienyl-5-(nona-9-carboxy-1(E),4(E)dienyl)catechol; and 4-(octa-2(Z)-enyl)-5-(deca-10-carboxy-2(Z),5(Z)-dienyl)catechol.

Compounds illustrative of Formula XXII are 7,7-difluoro-5(E),8(E),11(E),14(E)eicosatetraenoic acid; 7(L)-fluoro-5(E),8(E),11(E),14(E)eicosatetraenoic acid; 10,10-difluoro-5(E),8(E),11(E),14(E)eicosatetraenoic acid; 10(L)-fluoro-5(E),8(E),11(E),14(E)eicosatetraenoic acid; 13,13-difluoro-5(E),8(E),11(E),14(E)eicosatetraenoic acid; 13(L)-fluoro-5(E),8(E),11(E),14(E)eicosatetraenoic acid; 10-oxanorarachidonic acid; 10-azanorarachidonic acid.

A compound illustrative of Formula XXIII is 5-hydroxyborono-6(Z),8(E),11(E),14(E)-eicosatetraenoic acid.

A compound illustrative of Formula XXIV is 12-hydroxyborono-5(E),8(E),10(Z)14(E)eicosatetraenoic acid.

A compound illustrative of Formula XXV is 11-hydroxyborono-5(E),8(E),12(Z),14(E)-eicosatetraenoic acid.

A compound illustrative of Formula XXVI is 15-hydroxyborono-5(E),8(E),11(E),13(Z)-eicosatetraenoic acid.

F. <u>General Considerations relating to the Organic Compounds of this Invention</u>.

Compounds possessing substantially the same properties as those defined above, which are commercially available or can be prepared by means known to the art and are equivalents thereof, are those bearing one, two or more additional simple substituents, including but not limited to, halo, e.g., chloro; bromo; trifluoromethyl; nitro; sulfato; sulfonyloxy; carbo-lower-alkoxy, e.g., carbomethoxy; carbethoxy; amino; mono- and di-lower-alkylamino, e.g., methylamino; ethylamino; dimethylamino; methylethylamino; amido; cyano; etc.

Also included within the scope of this invention are pharmaceutically acceptable salts of the foregoing organic compounds, including those having alkali, preferably sodium and other alkali metal cations, alkaline earth metal cations, and other metal cations including zinc, aluminum, and cationic forms of the other metals listed above as cations of the multivalent metal salts with which the organic compounds of this invention are mixed. Preferred salts of the organic compounds are sodium and zinc salts.

A "contemplated class" of compounds for purposes of claims drawn to such class, may be a class consisting of any single compound embraced by the generic and subgeneric formulae herein, and/or named herein, a class consisting of a homologous series containing any such single compound, a class consisting of any such single compound and isomers thereof, a class consisting of any such single compound and analogs thereto, a class consisting of any combination of such single compounds, including structurally related compounds, and compounds having similar degrees of tested effectiveness.

G. Metal Salts.

The metal salts utilized in the mixtures of the present invention have as the metal component a multivalent metal. Examples of the metal portion of the salt include one or more of the metals, zinc, trivalent chromium, yttrium, divalent cobalt, nickel, magnesium, platinum, aluminum, monovalent copper, divalent copper, trivalent iron, trivalent cobalt, cadmium, antimony, mercury, rubidium, vanadium, and other rare earth metals. The salt is preferably a halide and the more preferred salt is a chloride.

Zinc salts, including halides, acetates, nitrates and sulfates, preferably halides, and more preferably chlorides, comprise highly preferred classes of metal

salts. The foregoing highly preferred salts, plus mono- and divalent copper, divalent cobalt, cadmium, antimony, trivalent iron and vanadium salts, preferably halides, and more preferably chlorides, comprise further preferred classes of metal salts. The most preferred metal salt is zinc chloride.

H. Methods of Preparation

When the mixture is to be applied topically as, for example, in the treatment of a tumor, wart or microbial affliction of the integument, it is preferred that the metal salt have the ability, though possibly only in higher concentrations than used in the mixtures of this invention, to exert an escharotic or keratolytic action.

In addition to the organic composition and the metal salt, the mixture can contain pharmaceutically acceptable chelators and/or antioxidants. Examples of chelators include urea, EDTA and its salts, diethylenetriamine tetraacetic acid (DTPA) and its salts, ethylenediamine-diacetic acid (EDDA) and its salts, nitrilotriacetic acid (NTA), ethylenediamine, salicylic acid, citric acid, gluconic acid, nucleic acid, oxalic acid, phosphates, sulfates, phospholipids, and amino acids. Examples of antioxidants include BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), ascorbate, citric acid, ethoxyguin, and alpha-tocopherol.

The mixture may also contain pharmaceutically acceptable carriers and/or diluents such as polyethylene glycol.

The organic composition may be mixed with a suitable solvent, the metal salt mixed with a suitable solvent, and the two solutions combined in appropriate amounts to achieve the desired concentrations. The preferred metal may be added in the form of its readily available salts such as acetates, or other aliphatic acid salts, while the preferred cation, e.g. chloride, may be added in the

form of its readily available salts such as sodium chloride. If complete solubilization does not occur, the mixture may be milled to obtain a fine suspension. When the formation of a metal chelate or complex is desirable, the order of mixing of ingredients and the pH may become critical, depending on the particular complex being formed, as exemplified below. A number of specific methods for preparation of particular mixtures are described in the Examples hereof.

The solvent for the organic composition will, of course, vary depending upon the particular composition. Examples of solvents for the compositions include absolute ethanol, glacial acetic acid, aqueous alkaline solutions, ethanol and dimethyl sulfoxide (DMSO). Some of the organic compositions are also soluble in other alcohols, ether, acetone, glycerol, propylene glycol, hot water, chloroform, glycerine, polyethylene glycol, etc.

The metal solution is of a salt or a chelate of the metal and it may be a mixture of different metal salts and/or chelates containing the desired metal ions and cations. It is preferred that the salt or chelate be water soluble. The solvent for the metal will generally be an aqueous solvent. The degree of reaction between the metal and the organic composition is dependent, _inter alia_, upon the affinity or stability constant of the specific metal ion for the particular organic composition at the particular pH of the system.

When chelates or complexes are utilized, compounds which can serve as counter-ligands are desirably provided so that discrete "molecular" entities rather than polymers of indeterminate length will form. Such counter-ligands include ethylenediaminetetraacetic acid (EDTA), ethylenediaminediacetic acid (EDDA), ethylenediamine, ammonia, amino acids and polyamines.

A preferred chelate is a chelate of NDGA and zinc. Such a complex may be formed by:

(1) mixing a zinc salt, preferably zinc chloride, with an appropriate amount of counter-ligand to fill all available coordination sites on the zinc, as may be readily determined by one skilled in the art. For example, when EDTA or EDDA are used as counter-ligands, an equimolar amount of zinc and counter-ligand should be used. When ethylenediamine is used as the counter-ligand, a molar ratio of ethylenediamine to zinc of 2:1 should be used. When ammonia is used as the counter-ligand, a molar ratio of ammonia to zinc of 4:1 should be used. The reaction may be conducted in any suitable medium, utilizing materials in which all reactants are readily soluble. The reaction mixture may be open to the air, but an inert atmosphere is preferred. pH should be adjusted to less than about 5 with a basic material, preferably an organic base, and more preferably excess counter-ligand. Following the preparation of the zinc/counter-ligand mixture, NDGA should be added utilizing an amount equal to half the zinc present. The pH is then further adjusted to greater than about 4 utilizing a base as described above.

(2) Utilizing the same ingredients and the same proportions as described above, NDGA is first mixed with counter-ligand in an inert atmosphere with pH adjustment as described above to greater than about 6. To this mixture is added the appropriate amount of zinc salt, and the pH is adjusted to greater than about 4.

Many of the organic compounds of this invention, including NDGA, are available commercially, and after purification as necessary, may be mixed with the metal salts as defined above.

The following organic compounds suitable for use in this invention may be prepared by the methods described in applicant's co-pending application number 436,444, the

contents of which are fully incorporated herein by reference: 1,4-bis (3,4-dimethoxyphenyl)-2,3-dimethyl butane-1,4-diol; 1,4-bis (3,4-dimethoxyphenyl)-2',3-butane; and the hydroxyphenyl variants thereof prepared by the demethylation process described in said application; and d,l-NDGA.

The following compounds suitable for use in this invention may be prepared by art recognized procedures:

1-(3,4-dihydroxyphenyl),4-phenylbutane; 1-(3,4-dihydroxyphenyl),4-(3,4,5-trihydroxyphenyl) butane; 1-(3,4-dihydroxyphenyl),4-(2,3,4-trihydroxyphenyl) butane; 1-(3,4-dihydroxyphenyl),4-(2,5-dihydroxyphenyl) butane; 1-(3,4-dihydroxyphenyl),4-(2,4-dihydroxyphenyl) butane; 1-(3,4-dihydroxyphenyl),4-(3,5-dihydroxyphenyl) butane; 1-(3,4-dihydroxyphenyl),4-(2,3-dihydroxyphenyl) butane; 1-(3,4-dihydroxyphenyl),4-(3-hydroxy,4-carboxylphenyl butane; 1-(3,4-dihydroxyphenyl),4-(3,5-ditertbutyl,4-hydroxyphenyl) butane; 1-(3,4-dihydroxyphenyl),4-(4-alkanylphenyl) butane; 1-(3,4-dihydroxyphenyl),4-diphenylbutane; 1-[4-(3,4-dihydroxyphenylbutanyl)] polyallyl alcohol; 1-4 bis(3,4-dihydroxyphenyl)-butyl benzene; alpha,omega-bis[4-(3,4-dihydroxyphenyl)-butanyl]-alkan-alpha,omega-diol; 1,1-bis[4-(3,4-dihydroxyphenyl)-butanyl] alkanol; 2-(2,5-dihydroxyphenyl),5-(3,4-dihydroxyphenyl) pentane; 1,4-bis(3,4-dihydroxyphenyl) pentane; 1-(3,4-dihydroxyphenyl),6,7-dihydroxytetralin; and diol analogs of the foregoing compounds having an OH substituent at the 4 position of the lignan chain, as well as analogs of all the foregoing having one or more hydroxy substituents on the rings replaced by methoxy substituents.

NDGA tetraacetate and NDGA-tetrapropionate may be prepared by respectively adding acetylchloride or propionylchloride dropwise to a cooled solution of NDGA in

pyridine. Other acetates, propionates, decanoates and long-chain esters may be prepared by analogous methods and methods known to the art.

Other compounds useful in this invention have been described in published literature and may be prepared by means known to the art.

H. Utility.

The novel mixtures of this invention are useful as antitumor agents, as antimicrobial, antiviral and antifungal agents, and as debriding agents for skin ulcers such as decubitus ulcers. They are useful in the treatment of herpes, and keratoses, especially actinic keratosis, and senile keratotic lesions. They are useful against a wide variety of premalignant and malignant skin tumors, basal cell carcinoma, squamous cell carcinoma and a diversified variety of melanotic lesions which are premalignant or malignant. The compositions are effective against mammalian tumors arising from all three embryonic tissue types, namely squamous cell carcinoma, e.g., lung carcinoma, arising from the ectodermal layer; adenocarcinomas, e.g., breast, renal and colon cancers, arising from the endodermal layer; melanoma and brain cancers, arising from the mesodermal layer and hematogenous tumors. They are also useful for the treatment of acne and warts. (As mammals may be mentioned, cat, dog, rat, horse, mouse, and monkey. The term "mammal" as used herein refers to lower animals and does not include humans.)

I. Dosages and Methods of Treatment.

The pharmacologically active mixtures of this invention should be present in amounts ranging from about 0.5 to 100 percent of a formulation. When applied topically, the drug will be contained in a pharmaceutically acceptable carrier, for example, a cream, ointment or solution. Polyethylene glycols are examples of suitable carriers for topical use. The frequency of application is dependent upon the conditions being

treated and the strength of the preparation. Generally, the compound will not be applied topically more often than once daily.

Generally, preferred molar ratios of the catecholic butanes to zinc with respect to two classes of tumors and exemplary application amounts/rate are shown in Table I.

TABLE I

Treatment/Use	Preferred Ratio of Catecholic Butane/Zinc	Exemplary Application Amount/Rate of Catechol/Zinc Composition
Pre-Malignant Tumors	1:5-5:1 1-10% cat. but./15-1% zinc	Apply topically 2-150 mg/cm^2 of tumor. Repeat application when amount of prior application falls below about 5 mg/cm^2. Wound may be dressed until healing is complete. Healing period may extend for several months.
Solid Epithelial Tumors	1:15-5:1 1-10% cat. but./30-1% zinc	Repeat daily as indicated by observation of tumor size reduction (i.e., if no reduction in size after 10 days, repeat 2-3 times daily; if reduction in size is served, after 10 days, repeat at daily intervals or sooner if reduction in size ceases to continue. Healing period may extend for several months. Alternatively, 0.1-20 ml. of composition may be injected intralesionally at the tumor site.

Typical formulations of the pharmaceutical compositions of this invention are set forth in Table II.

TABLE II

Application Form	Formulation	(Per 100 mgs.)	
Ointment	Zinc chloride	10.0	(preferred range: about 0.05-35)
	Catecholic butane	5.0	(preferred range: about 0.1-30)
	Peg 400	4.2	
	Peg 8000	61.7	
	Water	19.0	
	Ascorbic acid	0.1	
Gel	Zinc chloride	10.0	(preferred range: about 0.05-35)
	Catecholic butane	5.0	(preferred range: about 0.1-30)
	Standard denatured alcohol	10.0	
	Propylene glycol	22.5	
	Water	43.4	
	Non-ionic surfactant	6.0	
	Xantham gum	3.0	
	Ascorbic acid	0.1	
Cream	Zinc chloride	10.0	(preferred range: about 0.05-35)
	Catecholic butane	5.0	(preferred range: about 0.1-30)
	Ascorbic acid	0.1	
	Benzyl alcohol	5.0	
	Propylene glycol	23.0	
	Water	25.4	
	Stearyl alcohol	7.0	
	Cetyl alcohol	4.5	
	White petrolatum	13.0	
	Poloxyl-40 stearate	7.00	
Solid	Zinc chloride	5.00	(preferred range: 0.05-35)
	Catecholic butane	5.00	(preferred range: 0.1-30)
	Carnuba wax	8.88	
	Beeswax	13.32	
	Lanolin anhydrous	4.44	
	Cetyl alcohol	4.44	
	Ascorbic acid	0.10	
	Castor oil	57.70	
	Water	1.20	
Injectible Liquid	Zinc sulfate.$7H_2O$	2.00	(preferred range: 0.05-35)
	Catecholic butane	1.05	(preferred range: 0.1-30)
	Water	33.94	
	Glycerine	36.44	
	Glycine	1.52	
	Sodium ascorbate	0.05	
	Propylene glycol	25.00	

Sustained release topical preparations may also be used, including preparations releasing the metal salts and organic compounds at different rates.

Generally for topical use, an amount of the pharmaceutical preparation of this invention comprising between about 5-10 mg and about 500 mg per square centimeter of affected tissue is utilized. The area may be mechanically abraded before application of the preparation; or intratumor injection through the skin may be employed utilizing an amount of between about .01 ml and about 1.0 ml per cubic centimeter of estimated tumor volume. The affected area may beneficially be tape-stripped prior to application of the pharmaceutical preparation, and covered with a dermatological dressing after treatment.

The pharmacologically active mixtures of this invention can be introduced systemically by means known to the art in dosages adjusted to the pathology and its severity.

II. Zinc Potentiation of Organics for Tumor Reduction.

An embodiment of this invention involves the use of zinc salts, including nitrates, sulfates, acetates and halides, preferably zinc chlorides as potentiators of tumor-reducing properties of organic compounds. As used herein, "potentiation" is meant to indicate enhancement of the ability of a given amount of organic compound to reduce or eradicate tumors or retard tumor growth over and above the ability of the same amount of organic alone to reduce tumor size. This means, in the case of organic compounds previously known for their ability to reduce tumor size or retard tumor growth, that when the zinc salt is used in combination with such organics, a lesser dosage of organic is required to achieve the same effect.

Examples of such compounds are epipodophyllotoxin derivatives including epipodophyllotoxin beta-D ethylidene glucopyranoside (VP-16-213; etoposide) and epipodophyllotoxin beta-D thenylidene glucopyranoside (VM-26; teniposide); 4'-demethylepipodophyllotoxin; mitomycin C; daunomycin; cyclophosphamide; platinum cis-diaminedichloride; adriamycin; allopurinol; dithranol and diethylstilbestrol. This potentiation also occurs in the case of organic compounds not previously known for their ability to reduce or retard tumors, but whose tumor reducing or retarding abilities alone or in combination with zinc salts form part of the subject matter of this invention. Examples of such compounds are 3-tertbutyl phenol; 4-tertbutyl phenol; p-hydroxycinnamic acid; norisoguaiacin; d,l-NDGA; NDGA; azelaic acid; 1-(3,4-diacetoxyphenyl)-4-phenylbuta-1,3-diene; 1,4-bis(3,4-dihydroxyphenethyl) benzene. Some of these latter organics show no activity in reducing tumor size or retarding tumor growth when used alone, and require the presence of zinc salts to potentiate their threshhold activity.

The mechanism by which the zinc salt acts to potentiate the activity of organics for tumor retardation, reduction or eradication is not known, however zinc speeds penetration of the organic through the skin when it is topically applied. Zinc also causes the active organic to be retained in the skin longer than when zinc is not present. Thus for a given concentration of active organic, an effective amount can be kept at the site of skin pathology longer when a zinc salt is present than when it is not. Zinc may also aid in effecting penetration of the organic through cell membranes. The chelating effect of zinc may also further aid in carrying oxygen-containing organics into the cells. When the chloride anion is used it may add an independent potentiating

effect through super-oxide reactions into which it may enter.

The amount of zinc salt needed to achieve the potentiation effect is about 0.3% to about 30% of the total amount of zinc salt and organic compound. Zinc salts alone at low concentrations, e.g. less than about 0.7% zinc chloride, have not been found to have tumor retarding or reducing activity, but smaller amounts of zinc salts have the ability to potentiate known tumor retarding and reducing organics, such as the 4'-demethylepipodophyllotoxins, in the sense that too low a dosage of such compounds to be active is pushed over the threshold of activity by the addition of such small amounts of zinc salts.

The amount of zinc salt used must be at least enough to activate tumor-reducing activity of a lower-than-threshold dose of organic, or an otherwise inactive organic. This amount may vary from organic compound to organic compound and will depend on the amount of organic compound used. In the case of extremely toxic organics, where as low a dosage as possible is desired, or where a lower-than-threshold dosage of organics is required for some other reason, the least amount of zinc salt required will be the amount necessary to achieve activation. Where the organic is used at above threshold dosages, the lowest amount of zinc salt used would also be about that amount necessary to activate sub-threshold dosages. Where the organic is otherwise inactive, the lowest amount of zinc salt used would be that necessary to potentiate the smallest amount of organic capable of being so potentiated. In all cases, an upper limit of zinc salt needed is set by the amount which would cause the caustic effect of the mixture to substantially overshadow the cytotoxic effect of the organic, generally about 40% zinc salt concentration. It is generally preferred that the dosage of the organic compound be

optimized, and the ratio of zinc salt to organic used should be at least equal to that required for potentiation of the lowest possible dosage of the organic.

In general, the preferred amount of zinc salt, preferably zinc chloride, in accordance with the foregoing discussion is between about 1 and about 30 weight percent of the total preparation. Although other materials may be present in a pharmaceutical preparation containing an active organic and zinc salt, such as viscosity adjusters, stabilizers, preservatives, and the like, it is preferred that such additives not significantly compete with the active organic compound in providing coordination sites for the zinc ions.

III. Organic Tumor Reducing Compounds.

The compositions according to the invention, particularly nordihydroguaiaretic acid and related compounds, in preparations suitable for topical application or intratumor injection have been found to be effective at certain concentrations in reducing or eradicating tumors and/or stopping tumor growth. It has been found, surprisingly, that although NDGA stimulates tumor growth at low concentrations and is extremely toxic in concentrations high enough to cause tumor eradication after systemic administration, e.g. intraperitoneally or intravenously, effective concentrations can be administered without significant toxicity topically, and even by injection into tumors whose vascular blood supply connects to the rest of the organism, without significant toxicity. This compound appears to be selectively taken up and utilized by the tumor cells and possibly rapidly detoxified as it leaves the tumor bed. Similarly, the compositions according to the invention have been found to be effective and non-toxic for the treatment of the other treatments and diseases disclosed herein.

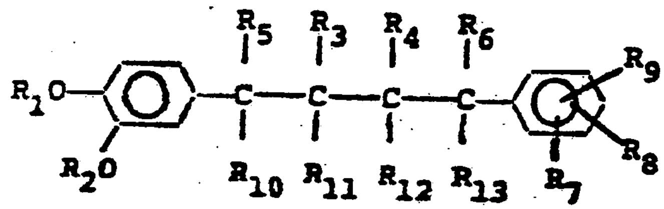
NDGA concentrations between about 4.0 and about 18.0 weight percent are effective against tumors when used topically or injected directly into the tumor. Higher concentrations, of about 8.8 to about 18 percent are

preferred, with about 16 to about 18 percent being most preferred.

The medicinal preparation should be dispersed in a suitable pharmaceutical carrier of sufficient viscosity to allow spreading of the preparation and good adherence to the membrane to which it is topically applied. A less viscous carrier, allowing injection via hypodermic syringe, is required when the preparation is to be injected into the tumor mass. Many suitable carriers are known to the art. A mixture of polyethylene glycols of about 400 and about 3350 molecular weight, adjusted to the desired viscosity has been found to be effective.

NDGA and its isomers, e.g. both meso and d,l-NDGA, as well as analogs such as norisoguaiacin, dihydroguaiaretic acid, 1-(3,4-dihydroxyphenyl)-2,3-dimethyl, 4-(3-methoxy,4-hydroxyphenyl) butane, 3'demethoxyisoguaiacin, and other compounds naturally occurring in Larrea, and pharmaceutically acceptable salts thereof with cations as defined above, are effective for use as described above, both with and without metal, e.g., zinc, ions.

With and without the presence of metal ions, the preferred catecholic butanes useful in the compositions of the instant invention are of the Formula



(II)

wherein R_1 and R_2 are independently H, lower alkyl or lower acyl;

R_3, R_4, R_5 and R_6 are independently H or lower alkyl;

R_7, R_8 and R_9 are independently H, hydroxy, lower alkoxy or lower acyloxy;

R_{10}, R_{11}, R_{12}, and R_{13} are independently H or lower alkyl.

Lower alkyl is intended to generally mean C_1-C_6 alkyl, and preferably R_3 and R_4 are C_1- C_3 alkyl. Lower acyl is intended to generally mean [C_1-C_6] acyl, with [C_2- C_6] being preferred. It will be appreciated by those skilled in this

art that Formula II is directed to both the phenolic compounds and the conventional esters and ethers thereof.

Illustrative classes of compounds within the scope of Formula (II) are those wherein:

a) one or more R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9, R_{10}, R_{11}, R_{12}, and R_{13}, are H, e.g., those wherein R_5, is H, R_5 and R_6 are H or R_5, R_6 and R_7 are H and R_8 and R_9 are OH or OR_1;

b) R_3 and R_4 each are CH_3 or C_2H_5 including those of a), especially those wherein R_5, R_6, and R_7 are H and/or R_8 and R_9 are OH and OR_1;

c) R_1 and R_2 are lower acyl, e.g., hydrocarbonacyl preferably, alkanoyl, e.g., acetyl, propionyl, etc., including those of a) and b);

d) R_1 and R_2 are alike and R_8 and R_9 are OR_1 including those of a), b) and c); and

e) The compound is in the form of a single optical isomer or a mixture of such isomers, e.g., a racemic mixture or diastereoisomers including each of a), b), c), and d).

As used herein, lower alkyl represents, inter alia, methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, tert-butyl, n-pentyl, isopentyl, n-hexyl, and the like.

Lower acyl represents groups having the general formula RCO-, e.g., acetyl (CH_3CO-), propionyl (CH_3CH_2CO-), butyryl ($CH_3CH_2CH_2CO$-), and the like. When the catecholic butane compound is named as a substituted phenyl, the corresponding groups are acetoxy (CH_3CO_2-), propionyloxy ($CH_3CH_2CO_2$-), and butyroyloxy ($CH_3CH_2CH_2CO$-).

Other useful compounds include 3-tertbutylphenol; 4-tertbutylphenol; p-hydroxycinnamic acid; norisoguaiacin; d,l-NDGA; 1-(3,4-diacetoxyphenyl)-4-phenylbuta-1,3-diene; and 1,4-bis-(3,4-dihydroxyphenethyl) benzene.

Additionally, certain aliphatic acids are useful as described above. Straight-chain acids of 6 or more carbons, preferably having carboxyl groups at both ends of the chain, are effective against solids tumors in mammals, including human breast tumors, when applied topically or by intratumor injection.

A preferred class of such dicarboxylic aliphatic compounds comprises alpha,omega-dicarboxylic acids, preferably C_7 to C_{14} dicarboxylic acids, and most preferably azelaic and dodecandioic acids. Such dicarboxylic acids are exemplified by: $HOOC-(CH_2)_5-COOH$; $HOOC-(CH_2)_6-COOH$; $HOOC-(CH_2)_7-COOH$; $HOOC-(CH_2)_8-COOH$; $HOOC-(CH_2)_9-COOH$; $HOOC-(CH_2)_{10}-COOH$; $HOOC-(CH_2)_{11}-COOH$ and $HOOC-(CH_2)_{12}-COOH$.

The following examples are included by way of illustration and not by way of limitation. Unless otherwise indicated, the nordihydroguaiaretic acid used in the instant Examples was the meso-isomer and is designated NDGA. Other isomers are indicated, e.g., d,l-NDGA.

EXAMPLE I

The catecholic butane 1-(3,4-dihydroxyphenyl)-4-(2,3,4 -trihydroxyphenyl) butane was prepared by the following procedure.

500 grams of 3,4-dimethoxydihydrocinnamic acid was suspended in 1.6 liters of methanol containing 250 ml of 2,2-dimethoxypropane. To this mixture was added

dropwise a solution made by adding 20 ml. of acetyl chloride to 400 ml of methanol. The resulting mixture was stirred overnight at room temperature and finally at reflux for one hour. The solvent was evaporated to give a syrup in quantitative yield, 533 g.

To 912 ml. of lithium aluminum hydride (1M in THF) was added dropwise 213 g. of 3,4-dimethoxydihydrocinnamic acid methyl ester dissolved in 900 ml of dry THF at such a rate as to maintain gentle reflux (5 hours). The reaction mixture was stirred overnight at room temperature, cooled in an ice bath and treated dropwise with ammonium chloride solution (saturated) (104 ml) over a two hour period. After stirring for several hours, the reaction mixture was diluted with 500 ml. of THF, filtered and the filtrate evaporated in a vacuum to give 160 g. (86%) of a light yellow oil.

3-(3,4-dimethoxyphenyl) propanol (202 g) was added to 218 ml of triethylamine in one and half liters of methylene chloride. This solution was cooled to -10°C in an ice salt bath and 87.6 ml. of methanesulfonyl chloride was added dropwise over a one and a half hour period while stirring rapidly. Stirring was continued for another hour and the mixture was washed with 700 ml. of ice water, 700 ml. of 3N hydrochloric acid, 700 ml. of saturated sodium bicarbonate and finally with 700 ml. of brine. The organic phase was dried with sodium sulfate and evaporated in a vacuum to give an orange oil in quantitative yield, 282 g.

3-(3,4-dimethoxyphenyl) propanol methanesulfonate, 282 g., (1.029 mol.); KBr, 282 g. (2.37 mol.) and dicyclohexano-18-crown-6, 19.2 g. (0.01515 mol.) were stirred in refluxing acetonitrile, 2.8 liters (dried over 3A molecular sieves) for 22 hours. The mixture was filtered and the filtrate evaporated in a vacuum to give

an orange oil, 267 g. The product could be purified by vacuum distillation at 0.5 mm Hg, b.p.=113-116°C.

3-(3,4-Dimethoxyphenyl) propyl bromide, 25.9 g., in 50 ml. of dry tetrahydrofuran (dried distillation from LAH) was placed in a dropping funnel. Magnesium powder, 2.5 g., and a trace of iodine was placed in a dry three neck flask with nitrogen inlet and reflux condenser. The reaction started upon addition of the liquid reactant and reflux was continued over a three hour period during which time the metal dissolved in the stirred solution. The reaction was cooled and the volume made up to 200 ml. to form a 0.5M solution in dry THF.

2,3,4-Trimethoxybenzaldehyde, 1.96 g. (0.01 mole), dissolved in 20 ml. of dry THF and 20 ml. of the 0.5M Grignard reagent from 3-(3,4-dimethoxyphenyl)propyl bromide in THF was added dropwise at ice temperature. The mixture sat over night at room temperature. The solution was evaporated in a vacuum and 20 ml. of ethanol was added carefully followed by excess sodium borohydride. Refluxing for a few minutes destroyed the yellow color of the small amounts of ketone and other unsaturated impurities formed from oxidation of the product. Most of the ethanol was evaporated and the residue partitioned between water and ether, 50 ml. of each. The ether phase was dried over sodium sulfate and evaporated to give 4.65 g. of a pale yellow oil.

The 4-(3,4-dimethoxyphenyl)-1-(2,3,4-trimethoxyphenyl) butanol, 3.65 g., was treated with excess sodium hydride, 1 g., and methyl iodide, one ml, in 25 ml. of dry dimethylformamide during one hour of stirring. Water was added carefully dropwise at first and finally 500 ml. of water was added. The product was extracted three times with 50 ml. of chloroform and the solvent evaporated to give a colorless crude oily product that

can be used in the next step without further purification.

About 100 ml of anhydrous ammonia was condensed into a three necked flask with a dry ice condenser and dry ice bath. The flask was protected from moisture with a soda-lime tube and flow of dry nitrogen. One gram of clean sodium metal was dissolved in the liquid ammonia and the whole of the crude product in 20 ml of dry tetrahydrofuran was added as quickly as possible. The dark blue solution was stirred rapidly for twelve minutes before enough methanol was added to destroy the blue color. Evaporation of the solvent under a vacuum gave a thick residue to which 500 ml. of water was added. The water solution was extracted twice with 50 ml. of chloroform that left three grams of oily residue on evaporation. Chromatography of this crude product on 300 g. of silica-gel using chloroform as an eluate gave 2.3 of pure 1-(3,4-dimethoxyphenyl)-4-(2,3,4-trimethoxyphenyl) butane (one spot on TLC).

A 1.15 g. sample of 1-(3,4-dimethoxyphenyl)-4-(2,3, 4-trimethoxyphenyl) butane was refluxed for nine hours in 50 ml. of 48% hydrobromic acid under an inert nitrogen atmosphere. Standing over the weekend allowed 641 mg. of tan product to settle out in the freezer. This material was recrystallized under inert atmosphere from methanol-water 1:20 to give light pink crystals, m.p.=165-167°C.

The following compounds were prepared by a similar procedure:

a) 1-(3,4-Dihydroxyphenyl)-4-(3,4,5-trihydroxyphenyl)butane;

b) 1-(3,4-Dihydroxyphenyl)-4-phenylbutane

c) 1-(3,4-Dihydroxyphenyl-4-(2,5-dihydroxyphenyl) butane;

d) 1,4-Di(3,4-dihydroxphenyl)-1,2,3,4-tetramethylbutane

e) 1,4-Di(3,4-dihydroxyphenyl)-2-methyl-3-ethylbutane

f) 1,4-Di(3,4-dihydroxyphenyl)-1-propyl-2-methyl-3-ethylbutane

EXAMPLE 2

To liquid ammonia (approximately 150 ml.) was added powdered ferric chloride, 150 mg., then small pieces of sodium, 1.53 g., were added and the blue color was allowed to dissipate over about a 20 minute period. To the resulting grey suspension of sodamide was added solid 3,4-dimethoxypropiophenone, 11.64 g., in small portions and the mixture was stirred for about five minutes. Solid alpha bromo-3,4-dimethoxy-propiophenone, 16.38 g., was then added in small portions to the grey-green mixture. After the mixture stirred for one hour 8. g. of ammonium chloride and 150 ml. of dichloromethane was added. The ammonia was allowed to evaporate and the mixture filtered while the solid residue was extracted twice with additional dichloromethane. Evaporation of the solvent to a small volume and dilution with methanol allowed crystallization of 19.75 g. of product.

The 2,3-bis(3,4-dimethoxybenzoyl)butane, 1.65 g., was not soluble in toluene, 25. ml, so enough dry tetrahydrofuran, 25. ml, was added to make the solution complete. An excess, 5. ml, of sodium dihydrobis(2-methoxyethoxy)aluminate (Vitride$_R$ 70% in toluene) was added and stirred at room temperature for several days. A saturated solution of sodium sulfate was added and the

some cases, the tumors were injected with the test compound or the control.

Almost all of the tumors demonstrated a significant reduction in size or were completely eliminated by the test compounds containing zinc chloride and NDGA.

Exemplary compositions of the mixtures are given in Table 2:

Table 3

Mixture	$ZnCl_2$	NDGA	EDTA	H_2O	PEGO
53	27.5	6.9	14.7	18.3	32.6
54	28	6.8*	14.7	18.2	32.9
55	16.4	6.9	8.6	18.0	32.2

* d,l NDGA

These mixtures were tested for their potential antitumor activities against B-16 melamonas grown in mice in accordance with the procedure discussed above. The results are given in Table 2a.

Table 3a

Mixture	n	T/C	Tumor Size (Control)	% Clear (Control)	% Survival (Control)
53	10	0	0 (575±270)	80 (0)	80 (60)
53	10	8	51±118 (711±286)	70 (0)	100 (100)
54	10	0	0 (711±286)	60 (0)	100 (100)
55	10	73	522±356 (711±286)	10 (0)	100 (100)

EXAMPLE 4

Fifteen older dogs having perianal adenomas were treated topically with the NDGA plus zinc salt ointment having a strength of 55% (w/w).

To 36.7 grams of powdered Larrea divaricata extract, containing 85% of weight NDGA, were added 24.5 grams of powdered rosehips and the mixture was mixed in a blender for 5 minutes. The blended mixture was then mixed with 100 milliliters of an aqueous solution containing 185.9 grams zinc chloride to form a paste. The paste was allowed to stand at room temperature for 24 hours. Thereafter, it was stirred and then placed in a screw-capped glass container. The container was placed in a humidified oven at 40°C for 5 days. This incubated paste was then suspended in 500 milliliters of water and shaken at room temperature for 24 hours on a reciprocating shaker. The zinc chloride extract

solution was then evaporated to near dryness on a rotary evaporator at 90°C under reduced pressure. A sufficient quantity of this dried zinc chloride extract was added to 120 grams of an ointment base consisting of 10% (w/w) stearyl alcohol and 90% (w/w) polyethylene glycol to obtain an ointment containing 70% (w/w) of the extract.

The normal treatment for such a condition is surgery; however, these older dogs were poor surgical risks. The tumor of each dog was biopsied and the ointment was applied topically into the biopsied incision. The duration of treatment varied depending upon the severity of the adenoma. Dogs with simple circumscribed adenomas required only one treatment. The dogs with more advanced adenomas generally required more than one treatment which were given three to five days apart. The treatment was successful in thirteen of the fifteen dogs. The treatment was not successful in two of the dogs which had extremely advanced cases of perianal adenomas.

EXAMPLE 5

Test compositions were prepared according to the following general method to test the activity of the compositions according to the invention against human breast adenocarcinoma, MX-1.

The NDGA, BHT (butylated hydroxytoluene), and Pego 400 were measured and mixed together with heating until melted and dissolved. Pego Base (50% Pego 400, 45% Pego 3350 and 5% stearyl alcohol) was prepared by mixing and heating the components together in a separate container until they dissolved. $ZnCl_2$ and EDTA were dissolved in water with heating and stirring in a separate container. The ingredients in each of the separate containers were added together in amounts needed to give the

cancer (glioma); melanoma; and colon cancer, CX-1. The test composition with the approximate wt/wt percentages given below was prepared according to the procedure previously described in Example 4. The control composition was Pego 400.

Ingredient	Test Composition 1	Control
BHT	0.16	-
EDTA	2.10	-
NDGA	0.66	-
$ZnCl_2$	4.26	-
H_2O	2.62	-
Pego Base	1.43	-
Pego 400	88.77	100

The composition was then tested for its effect on human tumors of varying origin implanted in athymic mice as previously described. Generally, there were ten mice in each group tested with Pego 400 control. Instances in which the number of mice varied are specifically indicated.

Results are given in Table 7.

TABLE 7

Tumor Type	Test Composition	Tumor Free at 60 Days	Premature Death	Tumor At Death	Recurrence
LX-1 (lung)	1	8	0	2	0
	control	0	0	5	0
MX-1 (breast)	1	8	0	2	1
	control	0	0	2	0
RX-1 (Renal)	1	8	1	1	0
	control	0	1	5	0
Glioma (Brain)	1	6	0	0	0
	control	0	0	2	0
Melanoma	1	10	0	0	0
	control	0	0	5	0
CX-1 (Colon)	1	8	1	2	0
	control	0	0	5	0

EXAMPLE 8

A number of catecholic butane compositions were formulated into test compositions according to the following general method, and tested for activity against human breast adenocarcinoma, MX-1.

Zinc chloride was dissolved in Pego 400 to prepare a stock solution. The amount of organic compound required to give the final concentration given below was separately dissolved in Pego 400.

The two solutions were mixed to give a final concentration in each test composition of zinc chloride at 0.69 wt/wt % and each organic compound at a molar concentration equivalent to 4.4 wt/wt % of NDGA.

The test compositions in Table 7 were tested for their effectiveness as antitumor agents against xenografts of the human breast adenocarcinoma, MX-1, grown in athymic mice. They were administered to five animals by intratumor injection. Animals were administered 0.05 ml of test composition unless indicated otherwise.

TABLE 8

Animal Organic Compounds	Tumor Free 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
Pego control	0	0	5	0
NDGA	4	1	0	0
d,l NDGA	5	0	0	0
NDGA Tetracetate	4	0	1	0
NDGA Tetrapropionate	4	0	1	1
1,4-bis(3' -methoxy-4' -hydroxyphenyl Butane	2	0	3	0
1,4-bis(3' -methoxy-4' -hydroxyphenyl)-2, 3-dimethyl butane	4	0	1	0
1-(3',4'-dihydroxyphenyl) -4-(2',3',4'-trihydroxy-phenyl)-butane	2	1	3	0
1-(3',4'-dihydroxyphenyl) -4(3',4',5'-trihydroxy-phenyl)-butane	3	0	2	1
1-(3',4',-dihydroxyphenyl) -4-(2',5'-dihydroxyphenyl) -butane	5	0	0	0
1-(3',4'-dihydroxyphenyl) -4-phenyl butane	3	1	1	0
1-(3',4'-dihydroxphenyl) -4-(2',4'-dihydroxyphenyl) -butane	2	0	3	3

EXAMPLE 9

Various zinc salts were tested in combination with NDGA to determine the effectiveness of the compositions according to the invention against xenografts of human breast adenocarcinoma, MX-1, grown in groups of five athymic mice.

The tumors were implanted subcutaneously in the left flank of the mice and the tumors were allowed to grow until they reached an approximate size of between 25 and 100 mm^2 (length x width). The mice were given a single 0.010 ml intratumor injection of the test composition. The concentration of the various metal salts in the test compositions was 0.73% (wt/wt) metal salt and 1.0% (wt/wt) NDGA, in a PEGO 400 base. The results of these test compositions are summarized in Table 8.

TABLE 9

Test Compound	Tumor Free at 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
$ZnCl_2$	4	0	3	3
$ZnSO_4 \cdot 7H_2O$	2	0	3	3
$ZnBr_2$	2	0	3	3
Zn Acetate $\cdot$ $2H_2O$	2	0	3	3
$Zn(NO_3)_2 \cdot 6H_2O$	3	1	1	1
$ZnCl_2$ (without NDGA)	1	0	19	19

In a separate trial, solubilized zinc gluconate demonstrated efficacious results in the *in vitro* inhibition of clonogenic human lung tumor cells (LX-T) when combined with NDGA.

EXAMPLE 10

This example describes the antineoplastic activity of

compositions containing NDGA and zinc ions in clinical studies on human patients with basal cell epithelioma.

Compositions as set forth in Table 9 suitable for topical application were prepared:

TABLE 10

Composition Compounds	A	B	C	D	E
zinc chloride	29.8	1.0	5.0	10.0	20.0
NDGA	4.6	4.6	4.6	4.6	4.6
EDTA	14.7	0.49	2.47	4.93	0
BHT	1.1	1.1	1.1	1.1	0
stearyl alcohol	0.5	0.5	0.5	0.5	0.5
H_2O	18.3	18.3	18.3	18.3	18.3
Pego 400	26.4	26.4	26.4	26.3	26.4
Pego 3350	4.5	4.5	4.5	4.5	4.5

The water was heated to about 80-90°C with stirring, and zinc chloride was added. The EDTA was next added with mixing until dissolved. In a separate container the polyethylene glycol 400 was heated to about 80-90°C with stirring, the NDGA was added thereto, then the BHT, and this mixture was added to the zinc chloride-EDTA solution with stirring. The entire mixture was then cooled to about room temperature and passed through a number 3 roller mill until smooth. The polyethylene glycol 3350 was then heated to about 80-90°C and the milled ingredients added thereto with mixing.

The surface of the lesions were tape stripped prior to each application. The test medication was applied directly to the lesion with a coating approximately 2mm thick, and covered with a dressing. After a minimum of

seven (7) days, a second application was applied at the discretion of the investigator. The dose ranged from 20-350 mg/cm^2 with as much as 500 mg/cm^2 utilized for deep tumors. To determine the effect of the test compound on the malignant neoplasma, an excisional biopsy was obtained 30 days after the initial treatment.

Of the fifty seven patients with basal cell epithelioma who were treated with compositions A, B, C or D, twenty showed negative biopsies, i.e., no evidence of tumor, at the conclusion of the treatment period.

EXAMPLE 11

Fifty-nine (59) human patients with actinic keratosis were treated with NDGA plus zinc containing compositions B, C, or D as in Example 9. The test medication was applied directly to the lesion with a coating of approximatley 2 mm and confined to the lesion margin. A dressing was applied to the lesion. A visual examination and measurement of the lesion was performed 7 and 14 days following the initial treatment. At the discretion of the investigator, a second treatment with the same test compound was applied. In order to determine whether the test compound eradicated the premalignant neoplasm, a punch biopsy was obtained 30-60 days after the initial treatment. If the biopsy report was negative, i.e., no tumor, the patient was examined every 6 months for a period of 12 months. If the biopsy continued to show evidence of actinic keratosis, the patient was withdrawn from the study and treated with conventional therapy.

The fifty nine (59) patients had a total of 61 lesions. After treatment with the NDGA plus zinc salt compositions, thirty two of the lesions showed negative biopsies, i.e., there was no evidence of actinic

keratosis.

EXAMPLE 12

Canine patients with various tumor lesions were treated with compositions A, C, D or E of Example 9. The animals were restrained from movement for two hours physically or with sedatives (e.g. 0.03 mg oxymorphone/lb.sq with atropine sulfate). After clipping, washing and measuring the tumor size, the skin surface was abraded until bleeding occurred. To enhance the penetration of the test compositions for large or subdermal tumors, a 20 or 22 gauge needle was used to puncture the tumor. After blotting the skin dry of blood, the tumor site was covered with a 1-2 mm coating of the test composition extending 5 mm peripherially. After 2 hours, the compound was wiped off and the area gently cleansed. The test composition was applied up to three times within a two-week interval or until the tumor cleared. The results of the canine studies are given in Table 11, and show that in canine patients, seven of the twenty four animals showed complete remissions, and another four showed partial remission.

concentrations desired and allowed to cool with vigorous mixing. Any further dilution to achieve desired wt/wt % was achieved by adding Pego 400. When an ingredient was omitted from a particular composition, the amount of the missing ingredient was supplied by adding additional Pego 400. Wt/wt % of compositions utilized in this experiment are given below.

Test Composition

Ingredient in wt/wt %	1	2	3
$ZnCl_2$	4.3	4.3	4.3
Purified water	2.6	2.6	2.6
EDTA	–	2.1	2.1
NDGA	0.66	0.66	0.66
BHT	0	0.66	0
Pego 400	91.04	88.28	88.94
Pego Base	1.4	1.4	1.4

The test compositions were tested in five athymic mice implanted with human breast adenocarcinoma, MX-1. Results are given in Table 4 and confirm the activity of these combinations of the phenolic butane, NDGA, and zinc ions.

TABLE 5

Test Composition	Tumor Free at 60 days	Premature Death	Tumor at Death	Tumor Recurrence
1	4	1	0	0
2	4	0	1	1
3	5	0	0	0

EXAMPLE 6

In order to demonstrate the activity and use of zinc

ions from other salts, two test compositions were prepared according to the procedure previously described. In these, the zinc chloride was replaced by zinc iodide and zinc bromide. Concentrations of the ingredients are given below in wt/wt percent.

Ingredient	Test Composition 1	Test Composition 2
BHT	0.65	0.72
EDTA	2.1	2.3
NDGA	0.98	1.1
ZnI_2	3.9	--
$ZnBr_2$	--	4.3
H_2O	2.6	2.9
Pego Base	1.4	0
Pego 400	88.37	88.68

The two compositions were tested for antitumor activity against human breast adenocarcinoma, MX-1, grown in five athymic mice as previously described. The results are given in Table 5.

TABLE 6

Test Composition	Tumor Free at 60 days	Premature Death	Tumor at Death	Tumor Recurrence
1	4	1	0	0
2	4	0	1	0

EXAMPLE 7

A test composition of NDGA plus zinc chloride was investigated for and found to possess antineoplastic activity against xenografts of the following human cancers: lung squamous cell carcinoma, LX-1; breast adenocarcinoma, MX-1; renal cell cancer, RX-1; brain

cancer (glioma); melanoma; and colon cancer, CX-1. The test composition with the approximate wt/wt percentages given below was prepared according to the procedure previously described in Example 4. The control composition was Pego 400.

Ingredient	Test Composition 1	Control
BHT	0.16	-
EDTA	2.10	-
NDGA	0.66	-
$ZnCl_2$	4.26	-
H_2O	2.62	-
Pego Base	1.43	-
Pego 400	88.77	100

The composition was then tested for its effect on human tumors of varying origin implanted in athymic mice as previously described. Generally, there were ten mice in each group tested with Pego 400 control. Instances in which the number of mice varied are specifically indicated.

Results are given in Table 7.

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Tumor Type	Test Composition	Tumor Free at 60 Days	Premature Death	Tumor At Death	Recurrence
LX-1 (lung)	1	8	0	2	0
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RX-1 (Renal)	1	8	1	1	0
	control	0	1	5	0
Glioma (Brain)	1	6	0	0	0
	control	0	0	2	0
Melanoma	1	10	0	0	0
	control	0	0	5	0
CX-1 (Colon)	1	8	1	2	0
	control	0	0	5	0

EXAMPLE 8

A number of catecholic butane compositions were formulated into test compositions according to the following general method, and tested for activity against human breast adenocarcinoma, MX-1.

Zinc chloride was dissolved in Pego 400 to prepare a stock solution. The amount of organic compound required to give the final concentration given below was separately dissolved in Pego 400.

The two solutions were mixed to give a final concentration in each test composition of zinc chloride at 0.69 wt/wt % and each organic compound at a molar concentration equivalent to 4.4 wt/wt % of NDGA.

The test compositions in Table 7 were tested for their effectiveness as antitumor agents against xenografts of the human breast adenocarcinoma, MX-1, grown in athymic mice. They were administered to five animals by intratumor injection. Animals were administered 0.05 ml of test composition unless indicated otherwise.

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Animal Organic Compounds	Tumor Free 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
Pego control	0	0	5	0
NDGA	4	1	0	0
d,l NDGA	5	0	0	0
NDGA Tetracetate	4	0	1	0
NDGA Tetrapropionate	4	0	1	1
1,4-bis(3' -methoxy-4' -hydroxyphenyl Butane	2	0	3	0
1,4-bis(3' -methoxy-4' -hydroxyphenyl)-2, 3-dimethyl butane	4	0	1	0
1-(3',4'-dihydroxyphenyl) -4-(2',3',4'-trihydroxy-phenyl)-butane	2	1	3	0
1-(3',4'-dihydroxyphenyl) -4(3',4',5'-trihydroxy-phenyl)-butane	3	0	2	1
1-(3',4',-dihydroxyphenyl) -4-(2',5'-dihydroxyphenyl) -butane	5	0	0	0
1-(3',4'-dihydroxyphenyl) -4-phenyl butane	3	1	1	0
1-(3',4'-dihydroxphenyl) -4-(2',4'-dihydroxyphenyl) -butane	2	0	3	3

EXAMPLE 9

Various zinc salts were tested in combination with NDGA to determine the effectiveness of the compositions according to the invention against xenografts of human breast adenocarcinoma, MX-1, grown in groups of five athymic mice.

The tumors were implanted subcutaneously in the left flank of the mice and the tumors were allowed to grow until they reached an approximate size of between 25 and 100 mm^2 (length x width). The mice were given a single 0.010 ml intratumor injection of the test composition. The concentration of the various metal salts in the test compositions was 0.73% (wt/wt) metal salt and 1.0% (wt/wt) NDGA, in a PEGO 400 base. The results of these test compositions are summarized in Table 8.

TABLE 9

Test Compound	Tumor Free at 60 Days	Premature Death	Tumor at Death	Tumor Recurrence
$ZnCl_2$	4	0	3	3
$ZnSO_4 \cdot 7H_2O$	2	0	3	3
$ZnBr_2$	2	0	3	3
Zn Acetate $\cdot$ $2H_2O$	2	0	3	3
$Zn(NO_3)_2 \cdot 6H_2O$	3	1	1	1
$ZnCl_2$ (without NDGA)	1	0	19	19

In a separate trial, solubilized zinc gluconate demonstrated efficacious results in the *in vitro* inhibition of clonogenic human lung tumor cells (LX-T) when combined with NDGA.

EXAMPLE 10

This example describes the antineoplastic activity of

compositions containing NDGA and zinc ions in clinical studies on human patients with basal cell epithelioma.

Compositions as set forth in Table 9 suitable for topical application were prepared:

TABLE 10

Composition Compounds	A	B	C	D	E
zinc chloride	29.8	1.0	5.0	10.0	20.0
NDGA	4.6	4.6	4.6	4.6	4.6
EDTA	14.7	0.49	2.47	4.93	0
BHT	1.1	1.1	1.1	1.1	0
stearyl alcohol	0.5	0.5	0.5	0.5	0.5
H_2O	18.3	18.3	18.3	18.3	18.3
Pego 400	26.4	26.4	26.4	26.3	26.4
Pego 3350	4.5	4.5	4.5	4.5	4.5

The water was heated to about 80-90°C with stirring, and zinc chloride was added. The EDTA was next added with mixing until dissolved. In a separate container the polyethylene glycol 400 was heated to about 80-90°C with stirring, the NDGA was added thereto, then the BHT, and this mixture was added to the zinc chloride-EDTA solution with stirring. The entire mixture was then cooled to about room temperature and passed through a number 3 roller mill until smooth. The polyethylene glycol 3350 was then heated to about 80-90°C and the milled ingredients added thereto with mixing.

The surface of the lesions were tape stripped prior to each application. The test medication was applied directly to the lesion with a coating approximately 2mm thick, and covered with a dressing. After a minimum of

seven (7) days, a second application was applied at the discretion of the investigator. The dose ranged from 20-350 mg/cm^2 with as much as 500 mg/cm^2 utilized for deep tumors. To determine the effect of the test compound on the malignant neoplasma, an excisional biopsy was obtained 30 days after the initial treatment.

Of the fifty seven patients with basal cell epithelioma who were treated with compositions A, B, C or D, twenty showed negative biopsies, i.e., no evidence of tumor, at the conclusion of the treatment period.

EXAMPLE 11

Fifty-nine (59) human patients with actinic keratosis were treated with NDGA plus zinc containing compositions B, C, or D as in Example 9. The test medication was applied directly to the lesion with a coating of approximatley 2 mm and confined to the lesion margin. A dressing was applied to the lesion. A visual examination and measurement of the lesion was performed 7 and 14 days following the initial treatment. At the discretion of the investigator, a second treatment with the same test compound was applied. In order to determine whether the test compound eradicated the premalignant neoplasm, a punch biopsy was obtained 30-60 days after the initial treatment. If the biopsy report was negative, i.e., no tumor, the patient was examined every 6 months for a period of 12 months. If the biopsy continued to show evidence of actinic keratosis, the patient was withdrawn from the study and treated with conventional therapy.

The fifty nine (59) patients had a total of 61 lesions. After treatment with the NDGA plus zinc salt compositions, thirty two of the lesions showed negative biopsies, i.e., there was no evidence of actinic

keratosis.

EXAMPLE 12

Canine patients with various tumor lesions were treated with compositions A, C, D or E of Example 9. The animals were restrained from movement for two hours physically or with sedatives (e.g. 0.03 mg oxymorphone/lb.sq with atropine sulfate). After clipping, washing and measuring the tumor size, the skin surface was abraded until bleeding occurred. To enhance the penetration of the test compositions for large or subdermal tumors, a 20 or 22 gauge needle was used to puncture the tumor. After blotting the skin dry of blood, the tumor site was covered with a 1-2 mm coating of the test composition extending 5 mm peripherially. After 2 hours, the compound was wiped off and the area gently cleansed. The test composition was applied up to three times within a two-week interval or until the tumor cleared. The results of the canine studies are given in Table 11, and show that in canine patients, seven of the twenty four animals showed complete remissions, and another four showed partial remission.

TABLE 12

	Test Composition	No. Animal Tested	Cure	Partial Effect	No Effect
Mast cell tumors	A	3	1	1	1
Mast cell tumors	C,D	6	-	2	4
Mast Cell tumors	E	2	1	-	1
Squamous cell carcinoma A	1	1	-	-	
Mammary Adenoma	A	2	1	-	1
Perianal Adenoma	A	7	1	1	5
Perianal Adenitis	A	1	-	-	1
Perianal Cyst (Benign)	A	1	1	-	-
Basal Cell Carcinoma	A	1	1	-	-
Totals		24	7	4	13

EXAMPLE 13

Equine patients with various tumor lesions were treated with compositions A, C, D, or E of Example 9. Melanoma, sarcoid and squamous cell carcinoma lesions were removed to skin level by surgical debulking; for papillomas, the lesion tips were removed. After hemostasis, the tumor site was covered liberally with the test compound extending 5 mm peripherially. Two weeks later, the crust was removed, the lesion area abraded and the test compound applied topically. After an additional two weeks, any crust was again removed from the lesion and the area abraded. The same test compound was again applied topically. Four weeks later, a biopsy of the lesion area was performed. The results of the equine studies in Table 12, show that NDGA plus zinc salt compositions show good activity against the tumor lesions in equine patients. The high activity of the composition against Papillomas, known to have viral

components, indicated the activity of these compositions.

TABLE 13

Lesion	Test Composition	No. Animals Tested	Cure	Partial	No Effect
Papillomas	A	4	4	-	-
Melanoma	A	4	3	-	1
Squamous Cell Car.	A	3	2	1	-
Sarcoid	A	5	4	1	-
Sarcoid	C or D	6	1	2	3
Sarcoid	E	5	5	-	-
		27	19	4	4

EXAMPLE 14

The *in vivo* antitumor effect of the interaction of NDGA and $ZnCl_2$ at various ratios was determined against MX-1 (human breast adenocarcinoma) cells.

Male or female athymic Balb/c mice, six to eight weeks of age and weighing 20 to 35 grams were used. MX-1 cells were cultured in the standard RPMI-1640 media and implanted subcutaneously in the flank of the nude mice in order to propagate the tumor line. Nude mice were implanted with 25 mg of the MX-1 solid tumor fragments. Tumors which reached the 25-100 mm^2 range were used for the experiment. 0.1 ml of the test compound was injected directly into the tumor. The tumors were measured periodically to determine their weight calculated by using half the product of the length times the width times the height of the tumor. The procedure was repeated at regular intervals until 60 days after

components, indicated the activity of these compositions.

TABLE 13

Lesion	Test Composition	No. Animals Tested	Cure	Partial	No Effect
Papillomas	A	4	4	-	-
Melanoma	A	4	3	-	1
Squamous Cell Car.	A	3	2	1	-
Sarcoid	A	5	4	1	-
Sarcoid	C or D	6	1	2	3
Sarcoid	E	5	5	-	-
		27	19	4	4

EXAMPLE 14

The *in vivo* antitumor effect of the interaction of NDGA and $ZnCl_2$ at various ratios was determined against MX-1 (human breast adenocarcinoma) cells.

Male or female athymic Balb/c mice, six to eight weeks of age and weighing 20 to 35 grams were used. MX-1 cells were cultured in the standard RPMI-1640 media and implanted subcutaneously in the flank of the nude mice in order to propagate the tumor line. Nude mice were implanted with 25 mg of the MX-1 solid tumor fragments. Tumors which reached the 25-100 mm^2 range were used for the experiment. 0.1 ml of the test compound was injected directly into the tumor. The tumors were measured periodically to determine their weight calculated by using half the product of the length times the width times the height of the tumor. The procedure was repeated at regular intervals until 60 days after

the initial treatment or all mice had died. Mice which showed no evidence of tumors were kept for 60 days to evaluate the potential for tumor recurrence, at which time tumor characteristics, if any, were recorded. Table 13 contains the results of the experiments using mixtures of NDGA and $ZnCl_2$ as well as the results of experiments with NDGA alone or with $ZnCl_2$ alone.

The effective doses (ED_x) at different response levels (x), determined in micromoles for $ZnCl_2$ alone, NDGA alone, and for the combination of $ZnCl_2$ in different molar ratios with NDGA are provided in Table 13.

The significant reduction in amount of either NDGA or zinc chloride required when given in combination is evident from the data. It is also seen that the total amount required for ED_x doses of the composition made up of NDGA plus zinc chloride is significantly less than the ED_x dose of NDGA or zinc chloride alone.

TABLE 14

MICROMOLES

	ED_{50}	ED_{75}	ED_{90}	ED_{95}
NDGA	13.6	25.7	48.3	74.3
$ZnCl_2$	15.7	22.2	31.6	40.1
NDGA (1:1)[1] +$ZnCl_2$	5.7	8.8	13.6	18.2
(1:2)[2]	4.6	6.2	8.4	10.4
(1:2)[3]	2.3	3.1	4.2	5.2

1 Calc'd as micromoles NDGA or $ZnCl_2$.
2 Calc'd as micromoles $ZnCl_2$
3 Calc'd as micromoles NDGA

EXAMPLE 15

Experiments were carried out indicating that zinc acts to stabilize the radical intermediate formed during oxidation of NDGA, thereby effectively stabilizing the NDGA and allowing it to exert its effect over a longer period of time before it is oxidatively inactivated.

Aqueous ethanolic solutions of NDGA with and without various metal salts at pH 4, 7, and 10 were analyzed in an ESR spectrometer for the presence of free radical ion.

The maximum peak height to minimum peak height of the ESR signal was measure over time. The reduction in ESR with time was used as a measure of free radical decay. The slope of free radical decay normalized to that of 3-hydroxytyrosine (DOPA) provided a measure of the relative rate constant of semiquinone free radical decay from NDGA.

The various rate constants, K_d, are given in Table 14.

EXAMPLE 16

To 36.7 grams of powdered Larrea divaricata were added 24.5 grams of powdered rosehips and the mixture was blended in a blender for 5 minutes. The blended mixture was then mixed with 100 milliliters of an aqueous solution containing 185.9 grams zinc chloride to form a paste. The paste was allowed to stand at room temperature for 24 hours. Thereafter, it was stirred and then placed in a screw-capped glass container. The container was placed in a humidified oven at 40°C for 5 days. This incubated paste was then suspended in 500 milliliters of triple distilled water and shaken at room temperature for 24 hours on a reciprocating shaker. The zinc chloride extract solution was then evaporated to near dryness on a rotary evaporator at 90°C under reduced pressure. A sufficient quantity of this dried zinc chloride extract was added to 120 grams of an ointment base consisting of 10% (w/w) stearyl alcohol and 90% (w/w) polyethylene glycol to obtain an ointment containing 70% (w/w) of the extract.

EXAMPLE 17

A sufficient quanitity of the paste of Example 17 was added to sterile deionized water to obtain a concentration of 10 grams per 100 milliliters of water. The aqueous mixture was thoroughly shaken for one hour on a reciprocating shaker, then the aqueous suspension was filtered through Whatman #1 filter paper in a Buchner funnel. The filtrate, an aqueous suspension, was used to irrigate wounds in the treatment of osteomyelitis.

EXAMPLE 18

Five selected human patients with osteomyelitis of duration of from several months to several years were treated topically with the solution of Example 17 and/or the paste of Example 16. In all instances, the osteomy-

elitis had been unresponsive to conventional treatment, and upon the application of the preparation, the patients received no other conventional therapy except as indicated. In some cases, the wounds were debrided, prior to the application of the preparation. Upon application of the preparation, most patients experienced pain and a burning sensation over the area which had been treated and some patients additionally experienced swelling and inflamation. One patient'experienced severe nausea after an application of the preparation.

Summaries, histories, and treatment are given below in Table 18. With respect to patient one, the disease process was so extensive that prior to treatment, a partial amputation of his foot was indicated. With respect to patient four, the disease process was so extensive as to cause the exposure of the extensor tendons which normally necessitates their cutting. Moreover, as a result of the destruction of the bones of the ankle and foot, the possibility of an ankle fusion was considered; however, neither of these procedures was required as the patient became ambulatory without the assistance of either a cane or crutches within six months of the beginning of the treatment with the preparation.

TABLE 18

Patient	Diagonosis	Culture	Previous Treatment	Duration of Condition	Number of Treatments	Time Required for Healing of Lesion
1 (62 year old male)	Chronic diabetic ulcer of left foot with osteomyelitis extending down to the metatarsal head capsule, involving the flexor tendon of the fourth toe	Hemolytic Staphylcoccus aureus coagulase positive	Antibiotics with no response	Several months	2:13 days apart	1-1/2 months
2 (59 year old male)	Chronic ulceration of lateral aspect of the proximal fibula	Staphylcoccus aureus coagulase positive	Multiple skin graftings; multiple antibiotics	Several years	3:19 and 23 days apart	3 months
3 (63 year old male)	Chronic osteomyelitis of left ankle and distal tibia	Hemolytic Staphylcoccus aureus coagulas positive	Recent treatment with Betadine soaks	35 years	4:over a 3-month period (first two with the solution and last two with the paste)	9-1/2 months for complete recovery

TABLE 1B (Cont)

Patient	Diagonosis	Culture	Previous Treatment	Duration of Condition	Number of Treatments	Time Required for Healing of Lesion
4 (70 year old female)	Ulcer of the left foot with necrosis, drainage, destruction of the bones of the foot and ankle initiated by a bite from a brown recluse spider	Hemolytic *Staphylcoccus aureus* coagulase positive	Antibiotics and soaks	7 months	2:5 days apart	1-1/2 months for lesion; after 6 months able to walk without crutches
5 (68 year old male)	Stasis ulcers of lower left extremity due to circulatory impairment	-	Steroid cream and ointment	unknown	2:9 days apart. Treated with a diuretic and soaks were applied to the area to reduce swelling apparently caused by the treatment.	2-1/2 months

EXAMPLE 19

Fifteen older dogs having perianal adenomas were treated topically with the ointment of Example 16 having a strength of 55% (w/w). The normal treatment for such a condition is surgery; however, these older dogs were poor surgical risks. The tumor of each dog was biopsied and the ointment was applied topically into the biopsied incision. The duration of treatment varied depending upon the severity of the adenoma. Dogs with simple circumscribed adenomas required only one treatment. The dogs with more advanced adenomas generally required more than one treatment which were given three to five days apart. The treatment was successful in thirteen of the fifteen dogs. The treatment was not successful in two of the dogs which had extremely advanced cases of perianal adenomas.

EXAMPLE 20

An incubated paste of rosehips, zinc chloride and Larrea divaricata prepared in accordance with the method of Example 16 was placed into gelatin capsules such that each capsule contained 200 mg of the paste. A patient with glioastrocytoma was treated orally with these capsules. Prior to this treatment the patient had a resistant tumor which displaced the cranium and protruded from the right lateral aspect of the skull; the protrusion measured 7 x 7 mm. The patient received 200 mg oral doses four times a day for a total daily dose of 800 mg. Observable and subjective improvement occurred within seven days; in 71 days the tumor had become cystic and lysed. The protuberance of the skull was reduced to near normal dimensions by repeated aspirations of the clear amber cystic tumor fluid. The patient has been maintained on the 200 mg capsules given four times daily and has remained symptom free for over 18 months.

EXAMPLE 21

Eleven cutaneous ulcers in eight human patients were treated with a formulation containing 5% zinc chloride and 4.6% NDGA w/w. If excessive necrotic material was present, debridement of non-viable and foreign material was performed either surgically or with wet-dry dressings prior to treatment.

The test compound was applied directly to the cutaneous ulcer in an amount sufficient to cover the visual margins of the ulcer. The treated ulcer was then covered with a loose dressing and the patient advised against washing the treated area for a reasonable period of time. A scab or crust was observed to form on the surface of the ulcer. Normally within two weeks the crust had loosened to where it was sluffed off or could be readily removed. It was observed that granulation of the tissue in the ulcer had occurred in those ulcers which slowed clinical improvement. A second treatment with the Compound was applied after removal of the crust. The patient was visually examined and the ulcer measured within two weeks after the initial treatment. Thereafter, the patient returned twice a month for two months for a visual examination and measurement of the ulcer. Of the eleven (11) treated lesions, seven (7) were clinically improved.

EXAMPLE 22

Six (6) Kaposi's sarcomas in human patients were treated with Compound A, (a formulation containing 29.8% zinc chloride and 4.6% NDGA w/w) which was applied directly to the lesion with a thickness of approximately 2 mm and confined to the visual margins of the lesion. The lesion was then covered with a dressing and the patient advised against washing the treated area for a reasonable period of time. The patient was visually examined 1, 2, 3, 7 and 14 days after the initial treatment. If possible, accurate measurements of the lesion were taken and recorded. A second application of Compound A was applied as deemed necessary. After 14 days, a biopsy was obtained if the lesion appeared clinically improved. If the biopsy continued to show evidence of Kaposi's sarcoma or if the lesion was not clinically improved by the 14th day after the initial treatment, the patient was withdrawn from the study. Due to the serious nature of the disease, the 14-day time period was arbitrarily chosen as the termination point in order to provide patients who had not clinically improved the opportunity to pursue other methods of treatment regardless of biopsy results.

EXAMPLE 23

Cultures of representative microorganisms which included Gram negative and Gram positive bacteria, yeasts and molds were prepared to assess the effect of composition A of Example 10, as well as its separate components, on the survival and/or growth of the microorganisms. The microorganisms and the culture media used are given below.

- *Streptococcus sp.*, Group C, ATCC 9342 (*Stp. Pyocenes*, Lancefield Group A).
- *Staphylococcus aureus* (penicillin sensitive), ATCC 9144
- *Staphylococcus aureus* (penicillin resistant), ATCC 13301
- *Escerichia coli*, ATCC 11229
- *Proteus mirabilis*, ATCC 4675
- *Mycobacterium smegmatis*, ATCC 20
- *Bacteroides fragilis*, ATCC 23745
- *Candida albicans*, ATCC 28366
- *Candida kruseri*, ATCC 2159
- *Trichophyton mentacrophytes*, ATCC 9533
- *Microsporum canis*, ATCC 9084

All of the bacterial species, including *M. smegmatis*, were found to grow well in tryptic soy broth with dextrose (TSB). Good growth was also obtained with the yeast species in this medium. Although the fungal species grew in TSB, they grew somewhat better in Sabouruad's broth (SAB), and for the broth dilution tests with *T. mentagrophytes* and *M. canis* Sabouruad's was used. For spore production the fungal species were grown on malt-soil extract agar.

A series of tests were devised to determine the effect of direct exposure of the microorganisms to the test compositions. The tests were conducted according to the following general procedure.

Sterile tubes of a growth medium (broth) appropriate for the bacteria, yeast, or mold under test were inoculated and allowed to grow until the tube exhibited the maximum turbidity that could be expected for the particular species. For most bacteria and yeasts this time was 24 hours. For molds the procedure was different in that the fungal species were inoculated onto the surface of a malt extract-soil extract agar slant and allowed to grow at room temperature until a heavy mycelial growth with heavy spore production was observed. At this time the spores were washed from the mycelia with sterile water and agitation using a vortex mixer. The spore suspension was filtered through four layers of sterile cheesecloth into sterile tubes. The spore suspensions were handled from this point in the same manner as broth suspensions of bacteria or yeasts.

One milliliter of the bacterial, yeast, or mold spore suspension was transferred to a sterile 12-ml glass, conical, centrifuge tube covered with a sterile cap and centrifuged at 3,000 rpm for 15 min. Centrifugation was done at room temperature using a benchtop, angle-head, clinical centrifuge (Clay-Adams). After the bacteria, yeast, or mold spores were pelleted, the supernatant fluid was decanted and the tubes inverted over paper saturated with a biocide placed in a bacteriological hood.

The pellets in the centrifuge tubes were then mixed with 1 gram of the undiluted test material and allowed to remain in contact for 2 hours at 37°C for the bacteria and yeasts and at 25°C for the mold spores. At the end of the contact time, the test mixture was diluted 1 to 10 with growth medium (TSB or SAB broth). Additional serial

dilutions were made from the initial dilution up to 1 x 10^{-9}. Each material was tested in triplicate. The controls, which consisted of the microbial cells incubated with 1 gram of mineral oil, were diluted in the same way. All dilutions of both test materials and controls were then incubated at an appropriate temperature of 37°C for bacteria and yeasts and 25°C for molds to allow for growth of any viable cells present.

All bacterial species except M. smegmatis were incubated for 48 hours; M. smegmatis was incubated for 7 days. Yeast tests were incubated 48 hours. Molds were incubated for 10 days. For a determination of growth response, growth in tubes containing test compositions was compared to the growth in a mineral oil control at an equivalent dilution. Growth was indicated by turbidity in the broth medium.

Results of the direct exposure tests are given in Table 23.

TABLE 23

Direct Exposure Tests
(Organism Exposed to the Composition
for 2 Hours Prior to Dilution in Test Broth)

	Test Broth Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
	pH of Test Broth		5.5	6.8	7.0	7.0	7.0	7.0	7.0	7.0
	Precipitation of Test Compound	heavy ppt	heavy ppt	ppt	slight ppt	-	-	-	0	-
Streptococcus pyogenes	Growth in Test Broth		0/3 [1]	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Growth in Mineral Oil Control		+ [2]	+	+	+	+	+	+	+
Staphylococcus aureus (penicillin sensitive)	Growth in Test Broth		0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Growth in Mineral Oil Control		+	+	+	+	+	+	+	+
Escherichia coli	Growth in Test Broth		0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	Growth in Mineral Oil Control		+	+	+	+	+	+	+	+

1. No growth shown in any of the three tubes at that dilution.
2. Indicates growth of cells occurred.

TABLE 23 (Cont.)

	Test Broth Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
	pH of Test Broth		5.5	6.8	7.0	7.0	7.0	7.0	7.0	7.0
	Precipitation of Test Compound	heavy ppt	heavy ppt	ppt	slight ppt	-	-	-	0	-
Mycobacterium smegmatis	Growth in Test Broth	-	0/3	0/3	0/3	0/3	0	0	0	0
	Growth in Mineral Oil Control	-	+	+	+	0	0	0	0	0
Bacteriodes fragilis	Growth in Test Broth	-	0/3	0/3	0/3	0/3	0	0	0	0
	Growth in Mineral Oil Control	-	+	+	+	+	0	0	0	0
Candida albicans	Growth in Test Broth	-	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0
	Growth in Mineral Oil Conrol	-	+	+	+	+	+	+	+	0
Microsporum canis	Growth in Test Broth	-	0/3	0/3	0/3	0/3	0	0	0	0
	Growth in Mineral Oil Conrol	-	+	+	+	+	0	0	0	0

EXAMPLE 24

Tests were conducted on the effect of the direct exposure of representative microorganisms to several separate components of the compositions of Example 10. Pego base alone was tested in one series of evaluations to determine whether or not inhibition by this carrier would have to be considered in evaluating the results of the individual ingredients dissolved in it.

In order to better approximate the effects of pego base in the Example 10 formulations, the amount of polyethylene glycol present in the formulation was calculated. The pure base material was then diluted with water to this concentration. Mineral oil was used as a positive control.

Nordihydroguaiaretic acid (NDGA) and desmethyl NDGA (DM-NDGA) diluted in pego base were also tested for inhibitory properties against representative gram-negative and gram-positive bacteria and yeasts covering the spectrum of microorganisms used in these tests.

The initial concentration of the compounds tested was equivalent to the amount present in the composition, and the general procedure outlined in Example 23 was followed. After a 2-hour exposure of the microorganisms to this initial concentration, progressive 1 to 10 serial dilutions of the mixture were made to assess viability of any microorganisms present. Results are shown in Table 24.

TABLE 24

Growth of Selected Microorganisms Following Direct Exposure Tests to NDGA, Desmethyl NDGA, PEGO

Microorganism Tested	NDGA 1/ micrograms/ml				DM-NDGA 1/ micrograms/ml				PEGO 1/ micrograms/ml				Mineral Oil micrograms/ml			
Concentration of Test Material	4,600	460	46	4.6	1,100	110	11	1.1	310	31	3.1	0.31	500	50	5.0	0.5
Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Streptococcus pyogenes	0	0	0	0	0	0	0	0	2^+	2^+	2^+	2^+	4^+	4^+	4^+	4^+
Escherichia coli	2^+ 2/	4^+ 3/	4^+ 4/	4^+	0	0	4^+	0	4^+	4^+	4^+	4^+	4^+	4^+	4^+	4^+
Staphylococcus aureus (Penicillin Resistant)	0	0	4^+	4^+	0	0	4^+	4^+	2^+	4^+	4^+	4^+	4^+	4^+	4^+	4^+
Candida albicans	0	0	2^+	2^+	±	1^+	3^+	3^+	3^+	3^+	3^+	3^+	4^+	4^+	4^+	4^+

1/ All test media at all dilutions were at pH 7.3.

2/ A heavy precipitate formed and bacterial turbidity was estimated; there was obvious growth with heavy production of gas.

3/ Moderate precipitate.

4/ Light precipitate.

EXAMPLE 25

A combination of EDTA (ethylenediaminetetraacetic acid) and zinc chloride in pego base at the concentration in which these components are present in composition A of Example 10 was tested for its effect on the viability of representative microorganisms. All organisms given in Example 23 were tested except Candida kruseri, and Microsporum canis.

The test procedure followed was that generally described in Example 23. The initial test composition of EDTA/zinc to which the microorganisms were exposed for 2 hours of direct contact had a pH of about 2.0. None of the organisms tested retained viability after exposure to this test mixture. All mineral oil controls showed abundant (4^+) growth.

In every test a heavy precipitate formed when the test mixture was diluted to 10^{-2}. The pH of the 10^{-2} dilution was 4.75. No precipitate formed at the 10^{-1} dilution (pH 1.8), the 10^{-3} dilution (pH 6.60) or the 10^{-4} dilution (pH 7.0).

EXAMPLE 26

A test was conducted to assess the growth of *Escherichia coli* and *Staphylococcus aureus* in broth containing composition A of Example 10, NDGA or desmethyl NDGA diluted in glycerol. Test parameters and results are given below.

TABLE 49

	Growth (48 hr at 37°C)	
	E. coli	*S. aureus*
Glycerol		
1 ml in 10 ml TSB	4^+	4^+
0.1 ml in 10 ml TSB	4^+	4^+
NDGA in Glycerol		
100 ppm (as NDGA) in TSB	3^+	0
1,000 ppm (as NDGA) in TSB	3^+	0
Desmethyl NDGA in Glycerol		
100 ppm (as DM-NDGA) in TSB	3^+	0
1,000 ppm (as DM-NDGA) in TSB	3^+	0
Compound A in Glycerol		
100 ppm	4^+	4^+ (4.6 ppm NDGA + 1.1 ppm BHT)
1,000 ppm	4^+	0 (46 ppm NDGA + 11 ppm BHT)

EXAMPLE 27

A series of broth dilution tests were conducted to assess the effect of composition A of Example 10; and its separate components on the growth of microorganisms. The individual test materials were incorporated into pego base at the concentration in which they are present in the composition for testing. EDTA and zinc chloride were tested together. Each original formulation was diluted 1 to 10 with growth medium, (usually tryptic soy broth with glucose), and subsequent 1 to 10 dilutions were made of the previous dilution usually up to 1×10^{-4}. This test was done with no consideration given to the solubility of the test material when diluted. In all cases, controls consisting of cells in mineral oil diluted in TSB were made to test the effect of the medium on growth. The pH determination of each series of materials was made by testing a duplicate set of tubes that were uninoculated. Each dilution tube containing 10 ml. test broth was inoculated with 0.1 ml. of a 24-hour culture of all test species except M. smegmatis and the mold species. Spore suspensions of fungi (10 days) were used to inoculate the tubes for testing effects on M. canis and T. mentagrophytes, and Sabouraud's broth was used for dilution because the fungal species grew somewhat better in this medium than in TSB. In general, a stationary phase culture of each test species was used.

Results of the broth dilution tests are given in Table 27. The pH values given in the tables apply only to the dilution shown.

The readings of turbidity in the growth media which indicate growth of the microorganism are rated from 0 = no growth, to 4+ = turbidity equal to the control. A 4+ reading for one microbial culture does not mean that the turbidity of that culture was the same as a 4+ reading

for any other culture. A 4+ reading means that turbidity in the tubes of a particular test was equal to the turbidity of the appropriate control at the dilution compared.

TABLE 27

Broth Dilution Tests

Test Microorganism	Test Compound	Compound A				Control Pego Base			
	Broth Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
	Broth pH	2.6	5.2	6.6	6.6	7.1	7.1	7.1	7.1
Streptococcus pyogenes		0	0	0	4+	4+	4+	4+	4+
Staphylococcus aureus (Pen. Resistant)		0	0	0	4+	4+	4+	4+	4+
Staphylococcus pyogenes (Pen. Sensitive)		0	0	0	4+	1+	4+	4+	4+
Escherichia coli		0	0	4+	4+	4+	4+	4+	4+
Proteus mirabilis		0	0	4+	4+	4+	4+	4+	4+
Mycobacterium smegmatis*		0	0	0	2+	±	4+	4+	4+
Bacteriades fragilis		0	0	0	0	4+	4+	4+	4+
Candida albicans		0	1+	4+	4+	2+	4+	4+	4+
Candida kruseri		0	0	2+	3+	4+	4+	4+	4+
Trichophyton mentagrophytes		0	0	2+	2+	4+	4+	4+	4+
Microsporum canis		0	0	4+	4+	not tested			

TABLE 27 (Cont.)

Broth Dilution Tests

Test Microorganism	Test Compound	NDGA in Pego Base				Desmethyl NDGA in Pego-Base				EDTA Zinc Chloride 1/			
	Broth Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
	Broth pH	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	1.8	4.75	6.6	7.0
Streptococcus pyogenes		2^+	3^+	4^+	4^+	0	4^+	4^+	4^+	0	0	0	4^+
Staphylococcus aureus (Pen. Resistant)		0	4^+	4^+	4^+	0	2^+	4^+	4^+	0	0	0	4^+
Staphylococcus pyogenes (Pen. Sensitive)		0	4^+	4^+	4^+	0	1^+	4^+	4^+	0	±	4^+	4^+
Escherichia coli		±	4^+	4^+	4^+	1^+	4^+	4^+	4^+	0	0	4^+	4^+
Proteus mirabilis		4^+	4^+	4^+	4^+	4^+	4^+	4^+	4^+	0	0	4^+	4^+
Mycobacterium smegmatis		0	0	4^+	4^+	0	1^+	4^+	4^+	0	0	0	2^+
Bacteriades fragilis		0	0	2^+	2^+	0	0	1^+	1^+	0	0	0	2^+
Candida albicans		1^+	2^+	3^+	4^+	1^+	2^+	4^+	4^+	0	0	4^+	4^+
Candida kruseri		0	0	4^+	4^+	0	2^+	3^+	4^+	not tested			
Trichophyton mentagrophytes		0	0	2^+	2^+	0^+	0^+	2^+	2^+	0	0	2^+	2^+
Microsporum canis		0	0	4^+	4^+	0	0	2^+	2^+	not tested			

1/ At the 10^{-2} dilution, pH 4.75 all tubes had a heavy precipitate. No precipitate formed at the other dilutions.

EXAMPLE 28

Compositions containing NDGA, zinc chloride or a combination of NDGA-Zn were tested for antimicrobial activity individually against seven gram positive and gram negative bacterias, yeasts and molds.

In a preliminary screening test, a vehicle containing 30% polyethylene glycol-200 (PEGO-200) + 0.1% sodium ascorbate in deionized water at various concentrations was shown to exhibit no inhibitory effect on microbial growth and was chosen as the diluent for the test compounds. Stock solutions of the test compounds in 30% PEGO-200/water were prepared at the following weight percent concentrations: 4.6% NDGA + 0.1% ascorbic acid; 5.0% $ZnCl_2$ + 0.1% sodium ascorbate; and 4.6% NDGA + 5.0% $ZnCl_2$ + 0.1% sodium ascorbate. Aliquots of the stock solutions were diluted 1:10 and 1:100 with the 30% PEGO-200 diluent. The stock solutions were further diluted 1:10 with Brain Heart Infusion Agar, which was melted at 45°C prior to the addition of the test solutions. The agar containing the test solutions was then poured into 50 x 90 mm petri dishes and allowed to dry for four hours at room temperature prior to inoculation.

All Brain Heart Infusion slants were started at 35°C anaerobically except for T. mentagrophytes at 27°C and P. acnes at 35°C anaerobically. Those microbial slants incubated at 35°C were subsequently transferred to new slants at 35°C and incubated at the same temperature. All slants were harvested with 1 ml saline containing 0.05% Tween-80 and diluted with saline in the following amounts to be used as working inocula: 1 ml each of E. Coli, P. aeruginosa, S. aureus and B. subtilis was diluted with 99 ml saline; 1 ml of C. albicans and P. acnes was diluted with 9 ml saline; 1 ml of T. mentagrophytes was left undiluted.

One drop of working inocula was added to the petri dishes containing the test compounds and allowed to

absorb into the agar. Uninoculated (control) and inoculated dishes were sealed and incubated in the dark for 5-7 days under the following conditions: P. acnes anaerobically at 35°C, T. mentagrophytes at 27°C and the remaining at 35°C aerobically. The plates were visually observed for microbial growth. Table 28A shows the dose levels and inhibitory effects of the test compounds. Table 28B provides a summary of the results with the test compounds showing the lowest dosage with complete inhibition.

TABLE 28A

% Active(s) in Agar	E. coli	P. aeru-ginosa	S. aureus	B. subtilis	P. acnes	C. albicans	T. mento-grophytes
0.46% NDGA	-	-	++	++	++	++	+
0.046% NDGA	-	-	++	++	++	+	-
0.0046% NDGA	-	-	-	-	-	-	-
0.5% $ZnCl_2$	++	++	++	++	++	++	++
0.05% $ZnCl_2$	-	-	-	+	+	-	-
0.005% $ZnCl_2$	-	-	-	-	-	-	-
0.46% NDGA + 0.5% $ZnCl_2$	++	++	++	++	++	++	++
0.046% NDGA + 0.05% $ZnCl_2$	-	-	++	++	++	+	+
0.0046% NDGA + 0.005% $ZnCl_2$	-	-	-	-	++	-	-
Vehicle Control (with 0.1% Sodium Ascorbate)							
0.2% Polyethylene Glycol-200	-	-	-	-	-	-	-
2.0% Polyethylene Glycol-200	-	-	-	-	-	-	-
20.0% Polyethylene Glycol-200	-	-	-	-	-	-	-

Results are found in triplicate samples:

++ = Complete inhibition of growth

\+ = Partial inhibition (some growth)

\- = Little or no inhibition (good growth)

EXAMPLE 29

A composition containing 5% NDGA plus 10% zinc chloride was tested for antiacne activity. Comedones were induced in both ears of rabbits by daily application of coal tar to the skin of the external ear canal. The right ear of each animal was treated with the test agent daily (5 days) for two weeks. The left ear served as the untreated control. Comedones in the test ears were small compared to those in the control ear. Horny material in the test ears was moderately reduced in 3 out of 5 animals. Peri-comedonal inflammation was significantly less in treated ears compared to test ears treated with vehicle alone.

While there have been described what are presently believed to be preferred embodiments of the invention, it will be apparent to a person skilled in the art that numerous changes can be made in the ingredients, conditions and proportions set forth in the foregoing embodiments without departing from the invention as described herein and as defined in the appended claims.

<u>What Is Claimed Is:</u>

1. An antitumor composition comprising the following components:

(a) zinc chloride; and

(b) nordihydroguaiaretic acid or a pharmaceutically acceptable salt thereof;

said components being present in amounts effective to prevent the replication of tumor cells.

2. The composition of Claim 1 in which zinc chloride is present in an amount of between about 1.0 and about 30.0 weight percent and nordihydroguaiaretic acid is present in an amount of between about 2 and about 18 weight percent.

3. The composition of Claim 1 in which zinc chloride is present in an amount of about 30 weight percent, nordihydroguaiaretic is present in an amount of about 4.6 weight percent, and the composition also includes ethylenediaminetetraacetic acid in an amount of about 14.7 weight percent and BHT in an amount of about 1.1 weight percent.

4. A method for treating conditions selected from the group consisting of basal cell epithelioma, actinic keratosis, cutaneous ulcers, and sarcoma comprising topically administering to a patient in need of such treatment a composition as described in Claim 1.

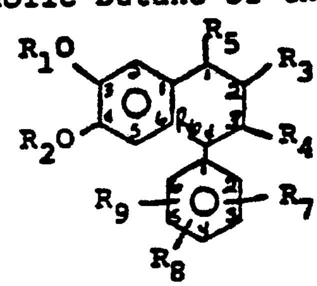
5. A method of treating a tumor selected from the group consisting of mast cell tumors, squamous cell carcinomas, adenocarcinomas, basal cell carcinomas, sarcoid tumors, and melanomas comprising topically administering to a mammal in need of such treatment a composition as described in Claim 1.

6. A method for preventing the replication of cells in solid human tumors selected from the group consisting of squamous cell carcinomas, breast adenocarcinomas, renal ~~cell~~ tumors, gliomas, melanomas, and colon tumors, comprising contacting said cells with a composition as described in Claim 1.

7. An antitumor composition comprising the following components:

(a) a metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) a catecholic butane of the formula:



I.

where R_1 and R_2 are independently H; 1-12 alkyl; 1-12 alkenyl; 1-12 alkoxy; 1-12 alkenoxy; $(CO)_n$ $(CH_2)_m$ $(CO_2)_p$ R_a, where n=0-1, m=1-4, p=0-1, and R_a is independently H, 1-12 alkyl, and 1-12 alkenyl; and glycoside moieties and R-substituted glycoside moieties wherein any of the hydroxyl hydrogens thereof may be replaced by R, with R being independently 1-2 alkyl, and 1-2 alkoxy; and taken together are methylene;

R_7, R_8 and R_9 may be attached to any separate location C_1-C_6 of their benzene ring, and are independently H; O; OR_1 (with R_1 defined as above); and when R_7 and R_8 or R_8 and R_9 are adjacent, taken together they may be methylene;

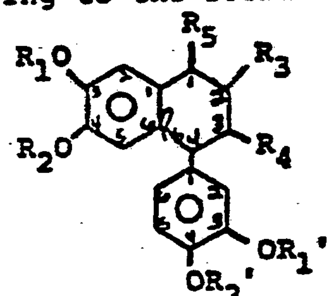
R_3 and R_4 are independently H, CH_3, C_2H_5, CHO and COOH; and

R_5 and R_6 are independently H, OH, OCH_3 and O;

and pharmaceutically acceptable salts thereof;

said components being present in amounts effective to prevent the replication of tumor cells.

8. The composition of Claim 1 in which the catecholic butane is selected from the group consisting of compounds corresponding to the formula:



II.

where R_1-R_6 are defined as in Formula I, and OR_1' and OR_2' are defined as R_1 and R_2, but may vary independently of R_1 and R_2; and

pharmaceutically acceptable salts thereof.

9. The composition of Claim 7 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

(b) The catecholic butanes are selected from the group consisting of 3,4,2',5'-quatrahydroxy-1,4-diphenylbutane; 2',3',4',3,4-pentahydroxy, 1,4-diphenylbutane; 3',4',5', 3,4-pentahydroxy, 1,4-diphenylbutane; and 1-(3,4-dihydroxyphenyl), 4-phenylbutane; nordihydroguaiaretic acid (1,4-bis-(3,4-dihydroxyphenyl) butane); dihydroguaiaretic acid (1,4-bis-(3-methoxy,4-hydroxyphenyl), 2,3-dimethylbutane); nordihydroguaiaretic acid tetraacetate (1,4-bis(3,4-diacetoxyphenyl),2,3-dimethylbutane; nordihydroguaiaretic acid propionate (1,4-bis-(3,4-dipropyloxyphenyl), 2,3-dimethylbutane); nordihydroguaiaretic acid glycoside (1-(3-hydroxy, 4-glucaryl-O-phenyl), -4-dihydroxyphenyl, 2,3-dimethyl-butane or 1-(4-hydroxy, 3-glucaryl-O-phenyl), 4-dihydroxyphenyl, 2,3-dimethylbutane); nordihydroguaiaretic acid glycoside tetraacetate (1-(3-hydroxy, 4-tetraacetoxyglucaryl-O-phenyl), 4-dihydroxyphenyl, 2,3-dimethylbutane or 1-(4-hydroxy, 3-tetraacetoxyglucaryl-O-phenyl), 4-dihydroxyphenyl, 2,3-dimethylphenylbutane); nordihydroguaiaretic

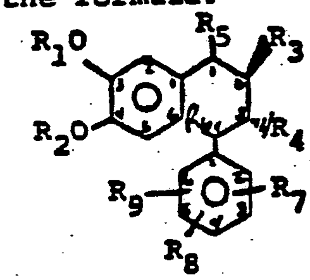
acid diphenoxyacetic acid diethyl ether (1,4-bis (3-dihydroxy) 4-diethylcarbonylmethoxyphenyl), 2,3-dimethylbutane or 1,4-bis (4-dihydroxy, 3-diethylcarbonylmethoxyphenyl), 2,3-dimethylbutane); nordihydroguaiaretic acid triphenoxyacetic acid diethylether (1-(3-hydroxy, 4-ethylcarbonylmethoxyphenyl), 4-(diethylcarbonylmethoxyphenyl), 2,3-dimethylbutane or 1-(4-hydroxy, 3-ethylcarbonylmethoxyphenyl), 4-(diethylcarbonylmethoxyphenyl), 2,3-dimethylbutane); nordihydroguaiaretic acid tetraethylhemisuccinate (1,4-bis(tetraethylhemisuccinylphenyl), 2,3-dimethylbutane; nordihydroguaiaretic acid tetramethylether diol (1,4-bis (3,4-dimethoxyphenyl) 2,3-dimethyl, 1,4-dihydroxybutane); nordihydroguaiaretic acid dimethylene ether dione (1,4-bis (3,4-dimethylenedioxyphenyl) 2,3-dimethyl, 1,4-dioxobutane); nordihydroguaiaretic acid tetramethylether dione (1,4-bis (3,4-dimethoxyphenyl), 2,3-dimethyl, 1,4-oxobutane); desmethyl nordihydroguaiaretic acid (1,4-bis (3,4-dihydroxyphenyl) butane); desmethyl nordihydroguaiaretic acid tetramethyl ether (1,4-bis (3,4-dimethoxyphenyl) butane; desmethyl dihydroguaiaretic acid; desmethyl nordihydroguaiaretic acid tetraacetate; desmethyl nordihydroguaiaretic acid propionate; desmethyl nordihydroguaiaretic acid glycoside; desmethyl nordihydroguaiaretic acid glycoside tetraacetate; desmethyl nordihydroguaiaretic acid diphenoxyacetic acid diethyl ether; desmethyl nordihydroguaiaretic acid triphenoxyacetic acid diethylether; desmethyl nordihydroguaiaretic acid tetraethylhemisuccinate; desmethyl nordihydroguaiaretic acid tetramethylether diol; desmethyl nordihydroguaiaretic acid dimethylene ether dione; desmethyl nordihydroguaiaretic acid tetramethylether dione; and pharmaceutically acceptable salts thereof.

10. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 9 in a pharmaceutically effective amount.

11. An antitumor composition comprising the following components:

(a) a metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) an optically active catecholic butane selected from compounds of the formula:



III.

where R_1 and R_2 are independently H; 1-12 alkyl; 1-12 alkenyl; 1-12 alkoxy; 1-12 alkenoxy; $(CO)_n$ $(CH_2)_m$ $(CO_2)_p$ R_a, where n=0-1, m=1-4, p=0-1, and R_a is independently H, 1-12 alkyl, and 1-12 alkenyl; and glycoside moieties and R-substituted glycoside moieties wherein any of the hydroxyl hydrogens thereof may be replaced by R, with R being independently 1-2 alkyl, and 1-2 alkoxy; and taken together are methylene;

R_7, R_8 and R_9 may be attached to any separate location C_1-C_6 of their benzene ring, and are independently H; O; OR_1 (with R_1 defined as above); and when R_7 and R_8 or R_8 and R_9 are adjacent, taken together they may be methylene;

R_3 and R_4 are independently CH_3, C_2H_5, CHO and COOH; and

R_5 and R_6 are independently H, OH, OCH_3 and O;
its antipodes and racemic mixtures thereof;
and pharmaceutically acceptable salts thereof;

said components being present in amounts effective to prevent the replication of tumor cells.

12. The composition of Claim 11 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

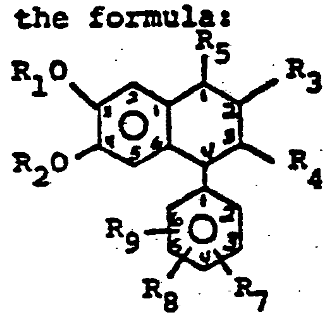
(b) the optically active catecholic butane is selected from the group consisting of d,l NDGA; d,l-dihydroguaiaretic acid; d,l-nordihydroguaiaretic acid tetraacetate, d,l-nordihydroguaiaretic acid propionate; d,l-nordihydroguaiaretic acid glycoside; d,l-nordihydroguaiaretic acid glycoside tetraacetate; d,l-nordihydroguaiaretic acid diphenoxyacetic acid diethyl ether; d,l-nordihydroguaiaretic acid trihenoxyacetic acid diethylether; d,l-nordihydroguaiaretic acid tetraethylhemisuccinate; d,l-nordihydroguaiaretic acid tetramethylether diol; d,l-nordihydroguaiaretic acid dimethylene ether dione; d,l-nordihydroguaiaretic acid tetramethylether dione; and pharmaceutically acceptable salts thereof.

13. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 12 in a pharmaceutically effective amount.

14. An antitumor composition comprising the following components:

(a) a metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) a tetralin selected from compounds corresponding to the formula:



IV.

where R_1 and R_2 are independently H; 1-12 alkyl; 1-12 alkenyl; 1-12 alkoxy; 1-12 alkenoxy; $(CO)_n$ $(CH_2)_m$ $(CO_2)_p$ R_a, where n=0-1, m=1-4, p=0-1, and R_a is independently H, 1-12 alkyl, and 1-12 alkenyl; and glycoside moieties and R-substituted glycoside moieties wherein any of the hydroxyl hydrogens thereof may be replaced by R, with R being independently 1-2 alkyl, and 1-2 alkoxy; and taken together are methylene;

R_7, R_8 and R_9 may be attached to any separate location C_1-C_6 of their benzene ring, and are independently H; O; OR_1 (with R_1 defined as above); and when R_7 and R_8 or R_8 and R_9 are adjacent, taken together they may be methylene;

R_3 and R_4 are independently CH_3, C_2H_5, CHO and COOH; and,

R_6 is H, OH, OCH_3 and O;

and pharmaceutically acceptable salts thereof; said components being present in amounts effective to prevent the replication of tumor cells.

15. The composition of Claim 11 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

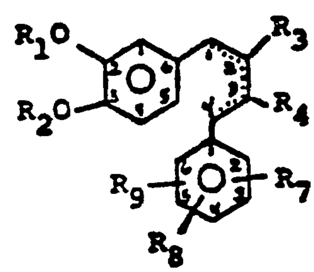
(b) norisoguaiacin; and pharmaceutically acceptable salts thereof.

16. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 14 in a pharmaceutically effective amount.

17. An antitumor composition comprising the following components:

(a) metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) catecholic butenes selected from compounds corresponding to the formula:



V.

where R_1 and R_2 are independently H; 1-12 alkyl; 1-12 alkenyl; 1-12 alkoxy; 1-12 alkenoxy; $(CO)_n$ $(CH_2)_m$ $(CO_2)_p$ R_a, where n=0-1, m=1-4, p=0-1, and R_a is independently H, 1-12 alkyl, and 1-12 alkenyl; and glycoside moieties and R-substituted glycoside moieties wherein any of the hydroxyl hydrogens thereof may be replaced by R, with R being independently 1-2 alkyl, and 1-2 alkoxy; and taken together are methylene;

R_7, R_8 and R_9 may be attached to any separate location C_1-C_6 of their benzene ring, and are independently H; O; OR_1 (with R_1 defined as above); and when R_7 and R_8 or R_8 and R_9 are adjacent, taken together they may be methylene;

R_3 and R_4 are independently H, CH_3, C_2H_5, CHO and COOH; and

R_5 and R_6 are independently H, OH, OCH_3 and O; and pharmaceutically acceptable salts thereof;

said components being present in amounts effective to prevent the replication of tumor cells.

18. The composition of Claim 17 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

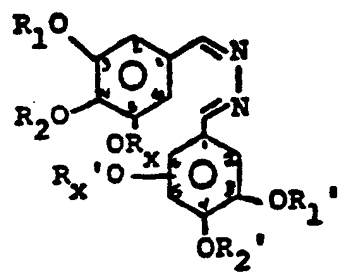
(b) the catecholic butene is selected from the group consisting of: 1,4-bis-(3,4-dimethylenedioxyphenyl) 1,3-butene and 2,3-bis (3,4-dimethoxybenzylidene) succinic acid; 1-(3,4-diacetoxyphenyl)-4-phenyl-buta-1,3-diene; and 1-(3,4-dihydroxyphenyl)-4-phenylbutadiene; and pharmaceutically acceptable salts thereof.

19. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 18 in a pharmaceutically effective amount.

20. An antitumor composition comprising the following components:

(a) a metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) an azine selected from compounds corresponding to the formula:



VI.

where R_1, R_2, R_1' and R_2' are independently H; 1-12 alkyl; 1-12 alkenyl; 1-12 alkoxy; 1-12 alkenoxy; $(CO)_n (CH_2)_m (CO_2)_p R_a$, where n=0-1, m=1-4, p=0-1, and R_a is independently H, 1-12 alkyl, and 1-12 alkenyl; and glycoside moieties and R-substituted glycoside moieties wherein any of the hydroxyl hydrogens thereof may be replaced by R, with R being independently 1-2 alkyl, and 1-2 alkoxy; and taken together are methylene;

and R_x and R_x' are defined as R_1 and R_1' respectively, and when $R_x'O$ and $R_2'O$ are adjacent they may be, taken together, methylene; and pharmaceutically acceptable salts thereof;

said components being present in amounts effective to prevent the replication of tumor cells.

21. The composition of Claim 20 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

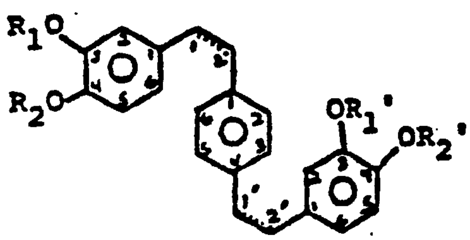
(b) the azine is selected from the group consisting of vanillin azine and syringaldazine; and pharmaceutically acceptable salts thereof.

22. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 21 in a pharmaceutically effective amount.

23. An antitumor composition comprising the following components:

(a) a metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) para-substituted benzenes selected from compounds corresponding to the following formula:



VII.

where R_1, R_2, R_1' and R_2' are independently H; 1-12 alkyl; 1-12 alkenyl; 1-12 alkoxy; 1-12 alkenoxy; $(CO)_n (CH_2)_m (CO_2)_p R_a$, where n=0-1, m=1-4, p=0-1, and R_a is independently H, 1-12 alkyl, and 1-12 alkenyl; and glycoside moieties and R-substituted glycoside moieties wherein any of the hydroxyl hydrogens thereof may be replaced by R, with R being independently 1-2 alkyl, and 1-2 alkoxy; and taken together are methylene, and there may be double bonds independently at 1-2 and 1'-2' of the chains; and pharmaceutically acceptable salts thereof; said components being present in amounts effective to prevent the replication of tumor cells.

24. The composition of Claim 23 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

(b) the para-substituted benzene compounds are selected from the group consisting of 1,4-bis-(3,4-dihydroxyphenethyl) benzene; 1,4-bis-(3,4-dimethoxyphenethyl) benzene; 1,4-bis-(3,4-dihydroxystyryl) benzene;

and 1,4-bis-(3,4-dimethoxystyryl) benzene; and pharmaceutically acceptable salts thereof.

25. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 24 in a pharmaceutically effective amount.

26. An antitumor composition comprising the following components:

(a) a metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) a bridged dicatecholic compound wherein the bridge is a 3-, 5- or 6-member carbon chain, and the catecholic hydroxyl hydrogens may be replaced with radicals which are, independently, 1-12 alkyl; 1-12 alkenyl; 1-12 alkoxy; 1-12 alkenoxy; $(CO)_n(CH_2)_m(CO_2)_pR_a$, where n=0-1, m=1-4, p=0-1, and R_a is independently H, 1-12 alkyl, and 1-12 alkenyl; and glucoside moieties and R-substituted glycoside moieties wherein any of the hydroxyl hydrogens thereof may be replaced by R, with R being independently 1-2 alkenyl and 1-12 alkoxy; and taken together are methylene; and wherein the bridging chain may bear 1-5 alkyl substituents; and pharmaceutically acceptable salts thereof;

said components being present in amounts effective to prevent the replication of tumor cells.

27. The method of Claim 26 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

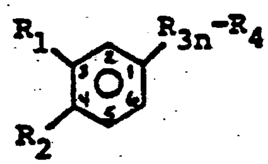
(b) the bridged dicatecholic compound is 1,6-bis(3,4-dihydroxyphenol) butane; and pharmaceutically salts thereof.

28. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 28 in a pharmaceutically effective amount.

29. An antitumor composition comprising the following components:

(a) a metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) phenolic acids and acidic anhydrides selected from the group consisting of compounds corresponding to the following formula:



VIII.

wherein R_1-R_2 are independently H, OH, 1-12 alkyl, 1-12 alkenyl, 1-12 alkoxy, 1-12 alkenyoxy, 1-12 alkylcarboxy, 1-12 alkenylcarboxy, or, taken together, are methylene dioxy;

$n = 0$-1

R_3 = 1-5 alkyl, 1-5 alkenyl, hydroxy-1-5-alkyl, hydroxy-1-5-alkenyl; oxy-1-5-alkyl; oxy-1-5 alkenyl, or oxo-1-5-alkyl, oxo-1-5 alkenyl; and

R_4 is an acid moiety, a 1-5 alkyl ester moiety, a 3-6 carbon dicarboxylic acid moiety, or a 3-6 carbon dicarboxylic acid anhydride moiety.

30. The composition of Claim 29 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

(b) the phenolic acid or acid anhydride is selected from the group consisting of 3,4-dihydroxybenzoic acid; ethyl 3,4-dihydroxybenzoate; cinnamic acid; mandelic acid; p-hydroxycinnamic acid; 3,4-dihydroxycinnamic acid;

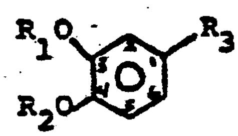
3,4-dihydroxyphenylacetic acid; 4-hydroxy, 3-methoxycinnamic acid; 2-(3,4-dimethoxybenzylidene) succinic acid; and 2-(3,4-dimethoxybenzylidene) succinic anhydride; and pharmaceutically acceptable salts thereof.

31. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 30 in a pharmaceutically effective amount.

32. An antitumor composition comprising the following components:

(a) a metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) a substituted catecholic compound selected from compounds corresponding to the following formula:



IX.

wherein R_1 and R_2 are independently, 1-12 alkyl, 1-12 alkenyl, or taken together are methylene; and

R_3 is 1-12 alkyl, 1-12 alkenyl, formyl, hydroxy-1-12-alkyl, hydroxy-1-12-alkenyl, oxo-1-12-alkyl, or oxo-1-12-alkenyl; and pharmaceutically acceptable salts thereof;

said components being present in amounts effect to prevent the replication of tumor cells.

33. The composition of Claim 32 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

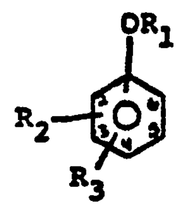
(b) the substituted catecholic compound selected from the group consisting of 4-methyl catechol; 4-tert-butyl catechol; 3,4-dimethoxyphenyl ethanol; 3,4-dihydroxybenzaldehyde; vanillin; 3,4-dimethoxyacetophenone; and 3,4-methylenedioxypropiophenone; and pharmaceutically acceptable salts thereof.

34. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 33 in a pharmaceutically effective amount.

35. An antitumor composition comprising the following components:

(a) a metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) phenolic compounds selected from compounds corresponding to the following formula:



X.

wherein R_1 is H or CH_3; and

R_2 and R_3 are independently H and 1-12 alkyl; and pharmaceutically acceptable salts thereof;

said components being present in amounts effect to prevent the replication of tumor cells.

36. The composition of Claim 35 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

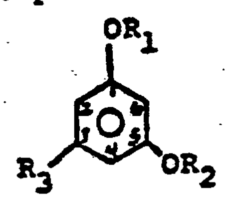
(b) the phenolic compounds are selected from the group consisting of phenol; 2-tertbutylphenol; 3-tertbutylphenol; 4-tertbutylphenol; thymol; and 2,3-dimethylphenol; and pharmaceutically acceptable salts thereof.

37. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 36 in a pharmaceutically effective amount.

38. An antitumor composition comprising the following components:

(a) a metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) a substituted resorcinol selected from the group consisting of compounds corresponding the following formula:



XI.

wherein R_1 and R_2 are independently H or CH_3; and

R_3 is 1-12 alkyl; and pharmaceutically acceptable salts thereof;

said components being present in amounts effective to prevent the replication of tumor cells.

39. The composition of Claim 38 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

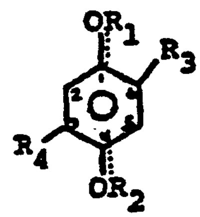
(b) the substituted resorcinols are selected from the group consisting of orcinol; 4-ethyl resorcinol; and olivetol; and pharmaceutical acceptable salts thereof.

40. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 39 in a pharmaceutically effective amount.

41. An antitumor composition comprising the following components:

(a) a metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) a quinone selected from compounds corresponding to the following formula:



XII.

wherein R_1 and R_2 are independently H and CH_3; and

wherein R_3 and R_4 are independently H and OH; and pharmaceutically acceptable salts thereof;

said components being present in amounts effective to prevent the replication of tumor cells.

42. The composition of Claim 42 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

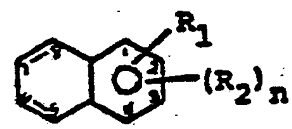
(b) the quinone compound is selected from the group consisting of hydroquinone and 2,5-dihydroxy-p-benzoquinone; and pharmaceutically acceptable salts thereof.

43. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 42 in a pharmaceutically effective amount.

44. An antitumor composition comprising the following components:

(a) a metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) a naphthalenic compound selected from compounds corresponding to the following formula:



XIII.

wherein n is 0-1;

R_1 and R_2 are independently OH, CHO, and COOH; and pharmaceutically acceptable salts thereof;

said components being present in amounts effective to prevent the replication of tumor cells.

45. The composition of Claim 44 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

(b) the naphthalenic compound is selected from the group consisting of 2,3-dihydroxynaphthalene; 1-naphthaldehyde; and 2-naphthaldehyde; and pharmaceutically acceptable salts thereof.

46. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 45 in a pharmaceutically effective amount.

47. An antitumor composition comprising the following components:

(a) metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) a flavone, flavanone, coumarin, quinizarin, ellegic acid, or purpurogallin bearing 0-2 substitutents per ring, said substituents being independently 1-12 alkyl, hydroxyl, 1-12 alkoxy, formyl, carboxyl and oxosubstituents; and pharmaceutically acceptable salts thereof;

said components being present in amounts effective to prevent the replication of tumor cells.

48. The composition of Claim 47 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

(b) the organic compounds are selected from the group consisting of flavone; flavanone; quercetin; 4-methyl esculetin; quinizarin; ellegic acid and purpurogallin trimethyl ether; flavanol (3-hydroxyflavone); (3,5,7-trihydroxyflavone); (3,3'4'-trihydroxyflavone); (3,4',5-trihydroxyflavone); datiscetin (2',3,5,7-tetrahydroxyflavone); fisetin (3,3',4',7 tetrahydroxyflavone); (3,3',4'-trimethoxy-7-hydroxyflavone); (3,3',4',7-tetramethoxyflavone); morin 2',3,4',5,7-pentahydroxyflavone); (8-hydroxykaempferol) kaempferol (3,4',5,7-tetrahydroxyflavone); quercetin (3,3',4',5,7-pentahydroxy-flavone); quercetagetin (3,3',4',5,6,7-hexahydroxyflavone); quercetin 3,3'-dimethylether; quercetin-7,3'-dimethylether; quercetin 3'-methylether; kaempferol (3,4',5,7-tetrahydroxyflavone); kaempferol-3,7-dimethylether; kaempferol

3-methylether; kaempferol 7-methylether; kaempferol 3,4'-dimethylether; quercetin 3,7,3',4'-tetramethylether; quercetin 3,7,3'-trimethylether; quercetin 7,3',4'-trimethylether; quercetin 3,7-dimethylether; luteolin 7,3'-dimethylether (3',4',5,7-tetrahydroxyflavone); luteolin 3'-methylether; apigenin 7-methylether (4',5,7-trihydroxyflavone); rutin (quercetin-3 rutinoside) 3,3'4',5,7-pentahydroxyflavone-3-rutinoside; chrysin (chrysoeriol 6,8-9-C-glucoside) 5,7-dihydroxyflavone; isoquercetin 3,3',4',5,7-pentahydroxyflavone-3-glucoside; kaempferol 3-O-rhamnosylglucoside (3,4',5,7-tetrahydroxyflavone); rhamnetin-3-o-rhamnosylglucoside (3,3',4',5-tetrahydroxy-7-methoxyflavone); myricetin (3,3',4',5,5'7-hexahydroxyflavone) (dihydromyricetin 3',5-dimethylether); hesperetin (herbacetin) 3,7-dimethylether; (3',5,7-trihydroxy-4'-methoxyflavanone); quercimeritrin $C_{21}H_{20}O_{12}$; 3,3',4',5,7-pentahydroxyflavone-7-D-glucoside [gossypitrin $C_{21}H_{20}O_{13}$] 3,7,3'-trimethylether; catechin (3,3',4',5,7-flavanpentol); gallocatechin; polydine; adzelechin, eupatilin and 4'-demethyl-eupatilin; delphinidin (3,3',-4',5,5',7-hexahydroxyflavilium); leuteoliniden; cyaniden (3,3',4,5,7-pentahydroxy flavylium); peonidum (3,4',5,7-tetrahydroxy-3'-methoxyflavylium); myrillidin; and enidin; and pharmaceutically acceptable salts thereof.

49. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 48 in a pharmaceutically effective amount.

50. An antitumor composition comprising the following components:

(a) a metal salt selected from the group consisting of salts of zinc, trivalent chromium, yttrium, divalent and trivalent cobalt, nickel, magnesium, aluminum, mono- and divalent copper, trivalent iron, cadmium, antimony, mercury, rubidium, vanadium and other rare earth metals; and

(b) an aliphatic acid, alcohol or aldehyde selected from the group consisting of dicarboxylic acids having four to twelve carbon atoms; and alcohols and aldehydes having four to twelve carbon atoms; and pharmaceutically acceptable salts thereof;

said components being present in amounts effective to prevent the replication of tumor cells.

51. The composition of Claim 50 in which:

(a) the metal salts are selected from the group consisting of nitrates, sulfates, acetates and halides of zinc, mono- and divalent copper, divalent cobalt, trivalent iron, antimony, cadmium and vanadium; and

(b) the aliphatic acids, aldehydes and alcohols are selected from the group consisting of adipic acid, azelaic acid, lauric acid, oxydiacetic acid, lauryl alcohol and octyl aldehyde; and pharmaceutically acceptable salts thereof.

52. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 51 in a pharmaceutically effective amount.

53. An antitumor composition comprising nordihydroguaiaretic acid in an amount of between about 16 and about 18 weight percent; and pharmaceutically acceptable salts thereof; in a suitable pharmaceutical carrier.

54. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 53 in a pharmaceutically effective amount.

55. An antitumor composition comprising an organic compound selected from the group including 3-tertbutylphenol; 4-tertbutylphenol; p-hydroxycinnamic acid; norisoguaiacin; d,l-NDGA; 1-(3,4-diacetoxyphenyl)-4-phenylbuta-1,3-diene; and 1,4-bis-(3,4-dihydroxyphenethyl) benzene; alpha,omega-C_7-C_{14} dicarboxylic acids; and pharmaceutically acceptable salts thereof; in a suitable pharmaceutical carrier.

56. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 55 in a pharmaceutically effective amount.

57. An antitumor composition comprising:

(a) an organic composition or a pharmaceutically acceptable salt thereof in at least a potentially pharmaceutically effective amount;

(b) a zinc salt selected from the group consisting of zinc nitrates, sulfates, acetates, and halides in an amount sufficient to potentiate the activity of the organic composition.

58. The composition of Claim 57 in which:

(a) the organic composition is selected from the group consisting of nordihydroguaiaretic acid; 3-tert-butylphenol; 4-tertbutylphenol; p-hydroxycinnamic acid; norisoguaiacin; d,l-nordihydroguaiaretic acid; azelaic acid; 1-(3,4-diacetoxyphenyl)-4-phenylbuta-1,3-diene; VP-16; VM-26; 4'-demethylepipodophyllotoxin; diethylstilbestrol; dithranol; mitomycin; daunomycin; platinum cis-diaminedichloride; allopurinol; and

(b) the zinc salt is zinc chloride.

59. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 58 in a pharmaceutically effective amount.

60. An antitumor composition comprising:

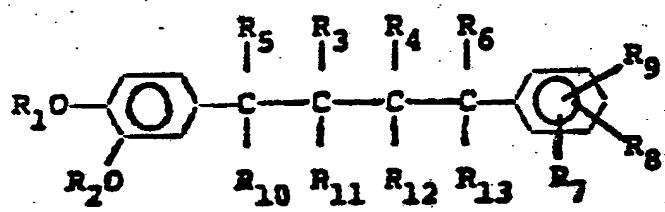
(a) zinc chloride; and

(b) an organic compound selected from the group consisting of: phenol; hydroquinone; 3,4-dihydroxy-cinnamic acid; cinnamic acid; 4-hydroxy-3-methoxy cinnamic acid; 4-tertbutyl catechol; 3,4-dihydroxy benzaldehyde; 4-methyl catechol; 4-ethyl resorcinol; desmethyl-N-tetra-methyl ether; quinizarin; olivetol; 2-tertbutylphenol; 3-tertbutylphenol; 4-tertbutylphenol; 2,3-dimethylphenol; thymol; 5-nitrovanillin; o-anisidine; purpurogallin trimethyl ether; vanillin; p-hydroxy-cinnamic acid; dihydroxynaphthalene; 2,5-dihydroxy-p-benzoquinone; orcinol; pentafluorophenol; picric acid; 3-(3,4-dimethoxy-phenyl) propylamine-N, N-acid . HCl; 3,4-dimethoxyphenyl-ethanol; 3,4-dimethoxyacetophenone; 3-(3,4-dimethoxy-phenyl) propylamine; 2,3-dihydroxybenzoic acid; 3,4-di-hydroxyhydro-cinnamic acid; 3,4-dihydroxyphenylacetic acid; 3,4-dihydroxybenzoic acid; 2,3-dihydroxybenzalde-hyde 3,4-methylenedioxypropiophenone; dl-NDGA; NDGA tetraacetate; 1,4-bis(3,4-dimethyleneoxyphenyl)butene; vanillin azine; syringaldazine; 2,3-bis(3,4-dimethoxy-benzoyl)butane; dihydroguaiaretic acid; norisoguaiacin; 2',3',4',3,4-pentahydroxy-1,4-diphenylbutane; 3',4',5',3,4-pentahydroxy-1,4-diphenylbutane; 1-(3,4-dihydroxyphenyl),4-(2,5-dihydroxyphenyl)butane; 1-(3,4-dihydroxyphenyl)-4-phenylbutane; calcein blue; quercetin; ellegic acid; 4-methylesculetin; flavanone; flavone; lauric acid; adipic acid; azelaic acid; oxydiacetic acid; 1-naphthalde-hyde; 2-naphthaldehyde; epipodophyllotoxin; epipodophyllo-toxin glycoside; VP-16; VM-26; lauryl alcohol; chloranil; n-octyl cyanide; octyl aldehyde; NDGA propionate; 3,4-di-hydroxybenzylamine-HBr; 2-aminophenol; 1,6-bis-(3,4-di-hydroxyphenyl)hexane; mandelic acid; 3-propyl catechol; 1-4-bis(dihydroxy, diethylcarbonylmethoxyphenyl), 2,3-di-methylbutane; 1-(3,5-dinitrophenyl)-4-(3,4-dimethoxy-phenyl)butene-1; 1,4-bis(3,4-dimethoxystyrl)benzene;

1,4-bis(3,4-dihydroxyphenethyl)benzene; beta-beta-bis(3,4-dihydroxyphenyl)-1,4-divinyl benzene; nordihydroguaiaretic acid diphenoxy acetic acid diethyl ester; nordihydroguaiaretic acid diphenoxy acetic acid triethyl ester; 1-(3,4-diacetoxyphenyl)-4-phenylbuta-1,3-diene; nordihydroguaiaretic acid tetraethyl hemisuccinate; nordihydroguaiaretic acid glucoside tetraacetate; nordihydroguaiaretic acid glucoside; 1-(3,4-dihydroxyphenyl)-4-phenylbutadiene; 2,3-bis(3,4-dimethoxybenzylidene)-succinic acid; 1-(3,5-ditrifluoromethylphenyl)-4-(3,4-dimethoxyphenyl)-butene-1; 1-(3,4-dihydroxyphenyl)-4-(3,5-ditrifluoromethylphenyl)butane; ethyl 3,4-dihydroxybenzoate; 2-(3,4-dimethoxybenzylidene) succinic acid; 2-(3,4-dimethoxybenzylidene)succinic anhydride; methyl carbamate nordihydroguaiaretic acid; ortho-para-desmethyl nordihydroguaiaretic acid; NDGA-tetrapropionate; acetoxyphenyl butadiene; NDGA-tetraacetate; 1-(3,4-dihydroxyphenyl)-4-phenylbutane; 1,4-bis-(3,4-dimethoxyphenyl)buta-1,4-diol; and pharmaceutically acceptable salts thereof; said components being present in amounts effective to prevent the replication of tumor cells

61. A method for preventing the replication of human breast adenocarcinoma cells in solid tumors comprising contacting said cells with a composition according to Claim 60 in a pharmaceutically effective amount.

61. A composition comprising at least 1 catecholic butane of the formula: wherein R_1 and R_2 are independently H, lower alkyl, or lower acyl;



R_3, R_4, R_5, R_6, R_{10}, R_{11}, R_{12} and R_{13} are independently H or lower alkyl;

R_7, R_8 and R_9 are independently H, hydroxy, lower alkoxy or lower acyloxy; and

ionic zinc.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US86/02547

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL(4): A61K 33/30; A61K 31/05
U.S. CL: 424-145; 514-734

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	424/145; 514/734;

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [6]

Chem Abstracts (1907-1986)
Guiaretic Acid
Dihydro and Derivatives; with or without Zinc Chloride

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	N, An Index Of Tumor Chemotherapy published (Mar. 1949), pages 10-12, 40 and 41, compound nos. 981-990, Dyer.	1-6
X	N, Cancer Research, Vol. 19, No. 10, Part 2 published Nov. 1959, pages 448-494 and 556 compound No. 16 440, Leiter et al.	53,54
X	U.S. A, 3,934,034 MANNING published 20 Jan. 1976	1-6,53 & 54
A	U.S. A, 4,094,994 SCHOENBERGER ET AL published 13 Jun. 1978	1-6, 53 & 54

* Special categories of cited documents: [15]
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
19 Feb. 1987	20 MAR 1987
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Jerome D. Goldberg

International Application No. PCT/US86 /02547

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	N, Chem. Abstracts, Vol. 96, (No. 14) Abst. No. 110,163v issed April 5, 1982 "Composition and Preparation of An Antimicrobial Agent" Abstracting French Demande (FR) Patent No. 2,482,860 Ladanyi	1-6

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ______, because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ______, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

See Attachment

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

   1-6, 53 and 54
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Attachment to PCT/ISA/210, Part VI.

Attachment to observations where unity of invention is lacking.

This International Searching Authority founds multiple inventions in this international application as follows:

Invention (I) Claims 1-6, 53 and 54

Invention (II) Claims 7-16 and 62

Invention (III) Claims 17-19

Invention (IV) Claims 20-22

Invention (V) Claims 23-25

Invention (VI) Claims 26-28

Invention (VII) Claims 29-31

Invention (VIII) Claims 32-34

Invention (IX) Claims 35-37 and 55-59

Invention (X) Claims 38-40

Invention (XI) Claims 41-43

Invention (XII) Claims 44-46

Invention (XIII) Claims 47-49

Invention (XIV) Claims 50-52